TEXAS REPORTS on BIOLOGY and MEDICINE

VOLUME 5, NUMBER 1 SPRING, 1947

Contents

PAGE

A PHYSIOLOGIC ANALYSIS OF CLINICAL EDEMA, *Eric Ogden and Eleanore Tripp* 1

EDEMA IN PREGNANCY, *Willard R. Cooke* 22

WILLIAM BUCHANAN LECTURE

INTRODUCTION, *Arild E. Hansen* 27

ADVANCES IN THE TREATMENT OF DIARRHEA IN INFANTS, *Daniel C. Darrow* 29

* * * * * * *

PYRIDINE-PROTEIN SILVER METHOD OF DEMONSTRATING NERVE FIBERS, *Glenn A. Drager* 55

ITHER OBSERVATIONS ON THE INTESTINAL BACTERIA OF GUINEA PIGS FED WITH P-AMINOBENZOIC ACID, *Dorothy M. Whitney* 63

SOME CHARACTERISTICS OF PENCILLIN-RESISTANT STAPHYLOCOCCI, *Edward H. Frieden, Helen R. Whiteley, and Chester N. Frazier* 74

OBSERVATIONS ON CELLS FROM ASCITIC FLUID CULTURED IN VITRO, *James B. Ivers and C. M. Pomerat* 92

THE DIAGNOSIS AND MANAGEMENT OF DIABETIC NEUROPATHY, *Raymond Gregory and E. L. Lindley* 112

CULTIVATION OF ENDOMETRIUM IN VITRO—A PRELIMINARY NOTE, *J. R. Valle and C. M. Pomerat* 145

CALLING ATTENTION TO 148

COPYRIGHT 1947
BY
THE BOARD OF REGENTS
OF
THE UNIVERSITY OF TEXAS

A PHYSIOLOGIC ANALYSIS OF CLINICAL EDEMA

ERIC OGDEN AND ELEANORE TRIPP*

INTRODUCTION

Edema, that is to say a great enough accumulation of tissue fluid to be recognized clinically, occurs frequently and in a wide variety of conditions. A logical analysis of the disturbed mechanisms underlying any particular instance of edema must be based on an understanding of the local function of tissue fluid and the way its production and reabsorption may be varied.

THE FUNCTION OF TISSUE FLUID

The statement of Claude Bernard to the effect that the constancy of the internal environment is the prime condition for free life has directed attention to the mechanisms for homeostasis which are developed to their highest degree in the mammal.

This internal environment is the tissue fluid in which the cells are bathed, and the constancy of its chemical and physical characteristics is paramount. The external environment of the body, particularly cold and an atmosphere not saturated with moisture, is constantly tending toward cooling and concentrating the tissue fluid. Further, the cells themselves are using the materials needed for energy, growth and repair. These materials pass by diffusion from the tissue fluid and the products of cell metabolism diffuse out into it. Metabolic activity also tends to raise the temperature of the tissue fluid. The protection against these changes in the internal environment arising from without and within lies solely with the mechanism whereby this tissue fluid is constantly removed and replaced by a standard tissue fluid at a rate great enough to prevent its composition from changing significantly.

From this point of view Bernard's statement might be modified to say that the normal operation of a cell is dependent

*From the Department of Physiology, The University of Texas School of Medicine, Galveston, Texas. Submitted for publication September 21, 1946.

upon a rapid turnover of tissue fluid in its immediate neighborhood. This makes it evident that edema, an accumulation of tissue fluid which may be thought of as stagnant, is in itself a bar to the normal working of cells.

Disturbances of function due to edema: There is no need to list all the changes which edema causes in the environment of cells; the majority of them are not accurately known, but oxygen deficit may be taken as a good example.

Since the cell depends for its oxygen supply upon diffusion from the tissue fluid immediately around it, a region of edema is a region of some degree of anoxia. Oxygen diffuses very slowly and is transported from the surface of the red cell to the active part of a tissue cell mainly by mechanical movement of the fluid in which it is dissolved and only to a limited extent by diffusion. Edema slows this movement and at the same time increases the distance through which oxygen must diffuse.

These circumstances which deprive the cell of its proper oxygen supply deprive it similarly of its other chemical requirements and of its facilities for eliminating waste products.

These are some of the reasons that the normal working of a cell is impaired in a region of edema and that edematous tissue is ill equipped to carry the extra load of healing. That edema delays healing and provides a culture medium for infecting organisms is well-known, but consideration of the mechanisms involved should direct attention to the wisdom of using this knowledge whenever edema is found.

Stagnation of tissue fluid also seems to favor fibrosis. Excessive fibrous reaction is a common accompaniment of oxygen deficit in other conditions besides edema, for example in avascular scar tissue, in the clubbed fingers of cardiac and pulmonary hypoxia or in the ill-ventilated parts of an emphysematous lung. In long-standing edema of the legs, from whatever cause, fibrosis further contributes to the poor healing ability and lowered resistance to infection.

Pigmentation of the skin is another sign of chronic anoxia and edema, but the mechanism involved is not clear. The pathologic results of this pigmentation are unknown but it is

a useful physical sign suggesting a history of local anoxia with the probability of fibrous scar tissue beneath the pigmented area of the skin.

Because of the damage it produces, *edema should be eliminated whenever it is found* except where there are very definite and important contraindications.

The Formation and Reabsorption of Tissue Fluid

In order to proceed effectively with the elimination of edema it is necessary to have a clear idea of why an excess of tissue fluid has accumulated and this knowledge must be dependent upon an understanding of the normal mechanisms of formation and removal of this fluid.

Our knowledge of tissue fluid formation was outlined by Starling half a century ago and should be briefly reviewed. According to his hypothesis, some of the fluid of the blood is forced out through the capillary wall into the tissue spaces; and under the influence of the pressure in the tissue space essentially the same material filters back into the capillaries in regions of lower intracapillary pressure and into the lymphatics.

The rate and direction of the flow into and out of the capillary system depend upon the balance of the forces involved and the ease of penetration of the filtrate through the capillary wall. The main force causing the outward flow is the intracapillary blood pressure which varies from moment to moment in different parts of the capillary system. This pressure is determined by the ease with which blood can leave the capillaries through the venules, veins and heart; the degree of vasodilation which allows a greater proportion of the arterial pressure to be delivered within the capillary; and to a very small extent upon the arterial blood pressure. As a result of catabolism, the break-down of large compounds into smaller ones, the filtration may be augmented by increased osmotic activity outside the vessels where metabolism is vigorous.

The pressure in the tissue spaces and the osmotic effect of the colloids which do not freely penetrate the capillary wall oppose the production of tissue fluid. The tissue pressure is

related to the amount of fluid formed and the space available for it. Where the subcutaneous fascia is tight as in the palm of the hand or the lumbar region a rapid rise of tissue pressure occurs when an excess of tissue fluid is formed; therefore these regions do not accumulate a large amount of fluid and show little edema. But in loose and readily distensible subcutaneous tissue like that around the eyes and on the dorsal surfaces of the hands much extra tissue fluid can accumulate with little rise in tissue pressure; and so there is little tendency for fluid to return to the vascular system and these regions become puffy in response to much smaller disturbances than other regions.

The osmotic force opposing formation and increasing reabsorption of tissue fluid depends upon the concentration of protein in the plasma, especially the albumin fraction. A fall in plasma albumin from nutritional deficiency, albuminuria or plasma dilution diminishes this osmotic force and produces edema.

The simplicity of the Starling-Heidenhain hypothesis makes it relatively easy to evaluate the immediate cause or causes of edema in most instances and so to guide treatment. The accumulation of clinical experience and recent experimental work strongly support this hypothesis but indicate that it does not give the whole story (Drinker). Although at present these observations cannot all be fitted together some of them may be considered.

The concept of the capillary wall as completely permeable to non-colloids and completely impermeable to colloids is no longer tenable, since protein, including globulin, is constantly found in tissue fluid though the amounts and proportions vary (Drinker and Yoffey). The penetration of proteins through the capillary wall is determined by the calcium content of the plasma, the pH and oxygen tension of the plasma and probably also of the surrounding tissue, and perhaps by certain ill-defined "toxic" states. Chambers and Zweifach have reported studies of the finer details of this field. Water and crystalloids pass mainly through the endothelial cells according to well-known physical principles, but the passage of pro-

teins and the diapedesis of cells out of the capillaries is probably through the intercellular cement substance whose properties are importantly modified by its chemical environment. Oxygen lack, lowered pH, and many of the other characteristic effects of metabolism apparently both dilate the capillary and increase its tendency to allow protein to pass. To date, experiments have not clearly separated these two phenomena which always occur together in nature.

Unfortunately the clearest picture of this phase in physiology has been obtained in the frog, an animal which is known to differ markedly from man with respect to the mechanics of its blood and lymph flow, the physico-chemical composition of its fluids and its general mechanisms for regulating its internal environment. At this time it seems unwise to draw a line between the normal behavior of the cell boundaries of the capillary and behavior which should be considered pathologic.

The same considerations are important in the reabsorption as in the formation of tissue fluid; but the larger particles, cells, debris, and probably protein molecules do not reënter the capillary but are returned to the blood stream by the lymphatic route after filtration and selective phagocytosis in the lymph nodes.

Physiologic Changes Causing Edema

Treatment and control of edema, as of all conditions, must be directed principally to the fundamental cause when this is known; symptomatic treatment may be used as a supplement and for its immediate effect. The disturbed mechanisms causing edema may be classified as protein dilution, raised intracapillary pressure, and toxic dilatation of the capillaries with increased capillary permeability. Lymphedema calls for separate discussion. In most clinical edematous states several of these causes are operating.

Edema caused by lowered plasma proteins: A lower concentration of plasma proteins diminishes the colloidal osmotic pressure within the capillary, thus favoring an increased formation of tissue fluid and diminishing the principal force

causing tissue fluid absorption. In this respect the albumin molecules, being of small molecular weight, are more osmotically active per gram of protein than are the globulins. Thus the albumin-globulin ratio must be regarded as important.

Edema with lowered plasma protein or disturbed albumin-globulin ratio must first be controlled by stopping the loss of plasma proteins which may be caused by hemorrhage, albuminuria, formation of ascitic or pleuretic fluid or by the loss of serum from surface exudates in burns or other extensive skin lesions. Whether the protein deficiency in the plasma arises from such losses or from nutritional or metabolic causes, protein should be immediately replaced by plasma transfusions, casein digests, or high protein food supplements as may be indicated in any individual case. The metabolic causes of diminished plasma protein are rarely susceptible of clear diagnosis and rational therapy but more usually improvement of the general health of such patients coincides with the rise of their plasma proteins to normal levels.

The plasma protein concentration, which is readily computed from the specific gravity measured on one drop of blood, is normally about 6–8%. When this falls to 5% in experimental animals edema appears and when the plasma protein level falls as low as 4% it is rarely possible to check the edema formation except by rapid intravascular infusion of large quantities of concentrated plasma. Experimental animals generally begin to recover from edema when the plasma proteins rise to this critical value of 5% whether as a result of intravenous treatment or of spontaneous regeneration of proteins by the liver. These experiments coincide very closely with the clinical observations on edematous patients when low plasma protein seems to be the sole cause of edema.

For these reasons it is wise to follow the plasma protein level closely in patients who are losing protein or who are likely to be deficient. In this way it is often possible to increase the vigor of treatment as the critical level is approached and to prevent or control edema. Lowering of plasma proteins is the principal or sole mechanism of edema formation in all edema of renal origin with the exception of acute glomerulo-

nephritis. In this disease the early accumulation of edema fluid of high protein content at a time when the plasma protein content is high suggests a change in the capillary permeability to protein and that the albuminuria is simply another expression of this wide-spread change.

In the strictly nutritional edemas proper care must include not only the emergency provision of proteins but the chemical stimulation and subsequent education of a proper appetite and feeding schedule and careful advice on the adjustments necessary to satisfy the nutritional requirements within the patient's means.

Edema from raised intracapillary pressure: Raised intracapillary pressure produces edema directly by raising the filtration pressure and increasing the amount of tissue fluid formed while at the same time raising the pressure which opposes the return of tissue fluid into the vascular system. The passive dilatation of capillaries which commonly accompanies raised capillary pressure increases the total surface through which tissue fluid formation takes place.

The commonest type of edema is due to increased capillary pressure arising from venous obstruction. This may be diminished by elevation of the part, by rest and by local cooling. The surgical care of obstructions as in varices, pelvic disease, axillary metastases, and the medical care of the obstruction which a failing heart offers to the return of blood represent fundamental treatments of the cause of such edema.

Toxic edema: Many chemical substances increase the permeability of the capillary and thus produce edema. These may be classified as extrinsic chemical poisons, products of tissue metabolism, and products of bacterial metabolism. Loss of function of a capillary always involves both dilatation and increase in permeability and critical experiments have failed to produce either phenomenon by itself.

Toxic derangements in organs other than the capillary may produce secondary edema; thus many toxic or infective kidney diseases by allowing the loss of protein produce edema which is indistinguishable in its mechanism from other edemas asso-

ciated with low plasma protein concentration. Whether nephritic edema is usually associated with a change in capillary permeability other than in the kidney is still uncertain.

Ingestion of abnormal amounts of sodium chloride and certain toxic states appear to cause water to go from the blood into the subcutaneous tissue rather than out through the kidneys. This condition is spoken of as "pre-renal deviation of water" and is found both normally and clinically in the presence of kidneys which are demonstrably capable of a proper excretion of both salt and water.

The treatment of toxic edema is unsatisfactory. Where there is an active bacterial process attempts to eliminate the source of infection by serological, chemotherapeutic or physical therapeutic methods are logical.

The emergency treatment of the edema itself in these cases is also unsatisfactory. Various diuretics are used to promote elimination of fluid through the urine, limitation of water and salt intake has been advocated and some attempts have been made to help the situation by substituting potassium for sodium as the base in all electrolytes taken in the food. In this connection hazards arising from the pharmacological action of potassium on the heart and perhaps the capillaries must be kept in mind. This is more fully discussed under the heading "Edema from renal diseases." The interrelationship between the water conserving activities of the pituitary gland and the salt regulating properties of the adrenal cortex probably play a role in the retention of salts characteristic of toxic edema but it has not been possible to use this information effectively in treatment.

Commoner Types of Edema Found Clinically

The rational approach to any case with edema depends on an understanding of the three physiological derangements which underlie all edema. These are: raised intracapillary pressure, increased capillary permeability, and lowered protein concentration in the plasma. Since almost every instance of edema involves more than one of these causes it seems worth-

while to analyze some of the commoner types of edema in this light.

Varicose Edema: The reversed flow of blood in the superficial veins of the patient with varices raises the pressure in the venules, obstructs the egress of blood from the capillaries and thus raises the intracapillary pressure causing edema. At first this is edema from raised filtration pressure in a completely uncomplicated form. It is however early and importantly complicated by the change in permeability of the capillaries which results from stagnant anoxia (Barcroft) within the vessels and from the tissue anoxia which the edema produces. A further contributing cause of varicose edema is found when thrombophlebitis or an infected ulcer complicates the picture by introducing marked arteriolar dilatation and some lymphatic obstruction.

The treatment of varicose edema has been on a rational basis for a long time. Attempts are made to diminish the venous stagnation and high intracapillary pressure by surgical or injection methods of interrupting the vessels believed to be causing the backflow. Rest with elevation lowers the intracapillary pressure and allows the edema fluid to gravitate away from the area it has already damaged. Many attempts, of which the Unna paste boot is the best-known, have been made to eliminate varicose edema by raising the tissue pressure and thus favoring reabsorption. One of the most successful of these techniques is the application of a tight non-distensible adhesive tape to the leg. This procedure has recently been described in detail by Ogden and Sherman.

Cardiac Edema: The heart which fails to force into the arterial system all the blood which is presented to its right atrium but allows some of it to accumulate on the venous side is producing a generalized obstruction to the venous return. This prevents the proper emptying of the capillaries and starts edema just as in varicose edema and with the same possibilities for the development of local anoxia. Cardiac edema, however, differs from varicose edema in that, being far more widespread, the possibilities for complicating factors are greater.

Pulmonary congestion leads to relative arterial anoxia and this accentuates the anoxia effects throughout the body. Anoxia, raised venous pressure, and perhaps a reflex vasoconstriction result in a failure of the kidney to form urine adequately and in a leakage of protein as albuminuria. The loss of protein and tendency to retain salts and water add to the edema-producing potential and perhaps some other substances which the kidney fails to excrete may further increase the permeability of capillaries in other tissues.

Inflammatory edema: The cause of inflammatory edema is two-fold. The break-down products of injured tissue, the products of infective agents and the metabolic products rapidly and continuously produced during the healing process cause the intense arterial and capillary dilatation characteristic of inflammation. These conditions tend to produce tissue fluid rapidly and so help to bring defense and repair materials to the source of inflammation. But they are apt to overload the facilities for the reabsorption which may be already impaired by the thrombus formation and hemoconcentration found in inflammation.

Certain special considerations arise in the treatment of inflammatory edema. The resolution of the inflammation or the removal of its cause will be followed by the subsidence of edema but in the presence of an active inflammatory agent attempts made to reduce edema are often contraindicated.

No simple means have been devised for hastening the absorption of tissue fluid from inflamed areas except through the judicious application of heat; in well-limited inflammation, gentle massage may sometimes help. The other way, an attempt to reduce edema by diminishing the rate of tissue fluid formation, is suspect when metabolic demands are high. Incisions for drainage of pus which commonly extend into the surrounding inflamed areas help to reduce edema by allowing the excess fluid to exude from the cut surfaces. The resulting diminution of tissue pressure allows the rapid formation of tissue fluid and flushes out that which has stagnated. This procedure is often helped by the use of agents such as magnesium sulfate and glycerin which promote the outward flow

of fluid from broken surfaces. There are many chemical hydrophile compounds, for instance wheat gluten, which will fix large quantities of watery fluids; the use of these compounds in the promotion of drainage has not yet received adequate trial. Yet another way to promote the absorption of tissue fluid and lessen edema, a method discussed in connection with varicose edema, is the application of tight dressings to raise the tissue pressure and thus facilitate the return of the fluid to the blood stream and promote a rapid turnover. The use of pressure in inflammatory conditions is open to a number of objections which are in some instances paramount; but this method has been recently used with success by Koch in the treatment of burns and by Trueta in other inflammatory conditions.

Edema from burns and frostbite: Edema after burns and frostbite is in many respects the same as inflammatory edema. The principal disturbance here is a marked arteriolar dilatation delivering blood at high pressure to the capillaries. The direct damage to the capillary by heat or cold and probably secondary chemical effects of the products of tissue destruction cause these capillaries to dilate and increase their permeability. It has been suggested that the pain in these conditions may contribute to the raised intracapillary pressure and rapid edema formation by a reflex sympathetic constriction of the veins. The rapid rate of exudation of fluid from the capillaries tends to block the capillaries with red cells. The breach in the skin or blister formation allows fluid to seep out from among the cells, lowers the tissue pressure and thus increases the local exudation and red cell accumulation. Secondary infection of burned tissue, which leads to disastrous systemic effects, produces local inflammation which accentuates the original injury.

Experimental studies by Montgomery on the rabbit's ear, an organ which closely resembles the human extremity in the structure, function, and mechanism of its vascular system, throw interesting light on edema of the extremities from burns. The two ears of a rabbit were simultaneously scalded and one

was immersed in running water at 18° C. The ear subjected to cold water produced noticeably less edema or none at all. The theoretical explanation of this, on which, however, no critical tests have been published, is that the cold water produces a marked contraction in the arteries of the ear and thus by protecting the affected capillaries against the delivery of the full aortic pressure diminishes the rate of edema formation. This seems a logical way for preventing edema in burns and in fact the healing of the ears thus treated was better than of the untreated ears.

That cool, but not freezing, temperatures will maintain arteriolar constriction even in the face of powerful dilating influences may well be important in the production of "immersion foot." This is an edematous condition known previously as "trench foot" but rediscovered during the war of 1939–45 and results from the prolonged immersion of the feet in cold though not necessarily freezing water (Ungley and Blackwood). The effect is most pronounced when the feet are constricted by tight shoes, boots or puttees. The clinical signs are gross edema, marked vasodilation, severe pain and after removal of the cold water and constricting footwear an intense and general dilatation of the blood vessels in the affected extremity lasting for weeks. It is possible that these symptoms result from extreme vasoconstriction which might keep the oxygen supply to the extremities so low as to produce edema by poisoning the capillaries from oxygen lack.

The treatment of all the conditions discussed in this section is highly controversial and is directed by considerations other than control of edema but it is significant that the treatment most strongly advocated for immersion foot is the exposure of the extremity to a continuous blast of cold dry air. This would tend to reduce the intracapillary pressure in the affected capillaries. The extremity is also elevated to remove the edema by gravity. In the modern treatment of burns a raising of the tissue pressure by plaster (Trueta) or by tight bandages (Koch) has been advocated; and the vigorous attempts usually made to raise the plasma volume by an infusion which is necessary to maintain the circulation against shock must also

help to some extent by maintaining the circulation and favoring the absorption of tissue fluid in areas where the blood was previously stagnant.

Edema from renal diseases: Acute glomerulo-nephritis shows many signs which indicate that it is a manifestation of acute generalized toxemia. Often this is of streptococcal origin and shows as do many other streptococcal diseases acute onset with marked fever, rapid recovery from the acute process with a delayed repair of the damage done during the disease and a liability to subsequent disease in the damaged organs. The tendency for the capillaries to leak in streptococcal diseases is fairly well recognized and in acute glomerulo-nephritis this is shown by the early onset of localized edema, by proteinuria and by the appearance of blood in the urine. This general picture makes it clear that the toxic changes in the capillaries are the primary cause of the edema in this disease and in the simple uncomplicated case they are probably the only cause since in spite of the raised arterial pressure there is no evidence that intracapillary pressure is usually raised nor does the plasma protein concentration commonly fall to the critical level.

Other renal diseases, however, are more complicated in the mechanism of the production of edema. The appearance of edema in chronic renal diseases usually runs parallel in time and intensity with the fall in plasma protein level which results from loss of protein in the urine.

As the chronic renal diseases progress other factors appear. The renal excretory failure probably tends to allow water to accumulate and also allows the accumulation of potassium and perhaps other "uremic" products which are toxic. How far these substances affect the permeability of the capillaries is unknown but they may contribute to edema in this way. Certainly in renal failure whether from kidney disease or cardiac insufficiency the plasma potassium levels are liable to go high enough to impair the action of the heart (Keith, Burchell and Boggenstoss) and thus to introduce an element of venous back pressure and raised capillary pressure. This sequence of

events is not obvious in clinical examination of the heart or veins until the diease is very far advanced but it seems likely that such changes though of a degree too small to measure may exert an influence on the production of edema since they are operating on all the capillaries of the body continuously over months and years.

Another complication in the progress of renal disease is the occurrence of hypertension. When hypertension occurs there are two possibilities for it to contribute to edema though their significance is difficult to evaluate and edema is not a prominent sign in hypertension without renal impairment. The vasoconstriction which is probably an essential feature in the production of hypertension may produce zones of relative anoxia and in these zones the capillaries may be expected to be more permeable. The increased load on the heart which sometimes progresses to produce heart failure and death must more commonly produce a sufficient degree of venous obstruction to contribute to edema formation. The significance of this factor, however, should be somewhat discounted since the back pressure and edema formation occur first in the pulmonary bed where recognition of relatively low grades of edema and congestion is easy. This factor, therefore, can usually be differentiated from factors producing systemic edema.

A number of studies have been made in an attempt to differentiate the pathologic physiology of edema as seen in various renal diseases but the above discussion is intended to collect together those factors which seem to be generally applicable to all renal impairment, primary or secondary, with the exception of the early phase of acute glomerulo-nephritis.

Eclampsia: The production of an abnormal "toxic" substance or some other derangement of the normal chemical functions of the placenta is the probable cause of the edema. Although there is no agreement as to the mechanism of eclamptic edema there is evidence to suggest that interference with the normal distribution of water and electrolytes and with the normal elimination by the kidney produces edema by the diversion of water and salts into the tissue spaces rather

than into the urine. On the other hand control of the intake of water or protein does not uniformly decrease the edema of the eclamptic patient but a rigid limitation of sodium chloride intake usually does so. This suggests that the key to this edema may be a deviation of sodium chloride and that the water merely follows the osmotic gradient passively.

The mechanism of this pre-renal deviation is unknown and must therefore be labeled toxic. It may be initiated by injection of vasopressin (postpituitary extract) into man and animals. Anselmino and Hoffman believed this substance to be an important part of the cause of eclampsia but subsequent workers have found evidence against this. More recently Shockaert and Lambillon have claimed that the pre-eclamptic woman is abnormally sensitive to the vascular effects of vasopressin.

In the pre-eclamptic state the increased pelvic tension and the very large vascular shunt through the uterine blood vessels must contribute to the formation of edema in the lower extremities as in fact they do in every pregnancy but in spite of the high incidence of eclampsia in women of particular body build, attempts to explain eclampsia on a mechanical basis have all been unsuccessful. Although edema and vascular changes are generalized in eclampsia, the prominence of the symptoms of intracranial edema are so important that one must look for some generally acting toxin (Page and Ogden).

Production of secondary edema as discussed under the heading of kidney disease may also occur during eclampsia and pre-eclampsia but since these conditions last for months rather than years the secondary effects are of less importance. Residual damage to the kidney after a toxic pregnancy may lead to delayed edema but for the purposes of this analysis this should be regarded as edema due to renal disease.

Edema during pregnancy: The nutritional, endocrine and hemodynamic alterations which constitute a part of normal pregnancy profoundly affect all edema producing situations. Willard R. Cooke has recently analyzed this aspect of the matter with his usual skill.

Angioneurotic edema and allied states: The conditions which produce edema cause accumulations of fluid which take on special characteristics in different regions of the body. Collections of fluid in the serous cavities and edema of the lungs are in essence similar to the edema elsewhere. The special conditions in these locations have led to a highly specialized literature in this field which will not be further discussed here.

In the skin and subcutaneous tissue edema may take on unusual appearances according to its exact location, distribution and the size of the affected area. Some of these are mosquito bites, urticaria, giant urticaria, angioneurotic edema and herpes. These all involve capillary dilatation, increased capillary permeability and arteriolar dilatation. The vascular changes may be the result of local chemical stimuli or may be mediated by the nervous system.

The hard wheal of a mosquito bite results from the outpouring of tissue fluid into a region of high tissue pressure because of the poisoning of the capillaries and reflex dilatation of the arterioles. This phenomenon was carefully investigated by Sir Thomas Lewis in his classical studies of the triple response of the skin to injury.

A similar reaction occurs more superficially in herpes and produces vesiculation. Here the stimulus seems, in part at least, to be antidromic vasodilator impulses which cause arteriolar dilatation and perhaps also capillary distention and increased permeability.

In giant urticaria and angioneurotic edema it is often difficult to separate a toxic (allergic or bacterial) factor from a psychogenic factor; but in any case these lesions are produced by localized capillary and arteriolar dilatation at different depths from the surface produced by chemical and nervous influences in varying proportions.

Understanding of these kinds of skin edema is limited by the dearth of exact experimental work in the field, but attention should be paid to the fundamental resemblance between simple edema and a wide variety of cutaneous lesions.

Lymphedema: Obstruction of the lymphatics may occur through the growth of malignant cells deposited in a lymph node, through pressure on the lymphatics from without or from the results of either acute or very slow inflammatory processes in the lymph nodes or in the main lymphatic channels. Experimental evidence shows very free exchange of fluid between the lymph vessels and the tissue spaces so that they behave physiologically as open-ended vessels. Some authors regard the composition of lymph as identical with that of tissue fluid.

The lymphatics are, however, very richly equipped with valves and therefore fluid once having moved into lymphatic vessels can progress only centrally. Obstructed lymphatic vessels become distended with fluid whose location is strictly fixed by lymphatic valves and gives the tissue the hard brawny feeling of non-pitting edema. Obstructive lymphedema disappears rapidly if its cause is removed; but lymphedema from chronic inflammation is highly refractory to treatment. Acute lymphatic infection is occasionally followed years after it has become quiescent by obstinate lymphedema. Although the fluid in lymphedema is fixed and cannot be moved by the pitting test, firm pressure for a long time on an area with lymphedema will cause the excess lymph to move on and thus reduce the edema at least while the pressure is maintained. This treatment has its place in elephantiasis and other lymphedema of the leg since reduction of the weight of the leg is useful and perhaps the removal of stagnant fluid leads to the cessation of the production of fibrous tissue or even to its absorption. More radical treatment of lymphedema has met with indifferent success. Neither the Kondoleon operation which aims at the removal of large masses of lymph-bearing subcutaneous tissue nor the surgical establishment of alternative lymph channels around regions of obstruction have proved thoroughly effective even in the hands of the most successful surgeons. Acute inflammatory conditions affect the lymphatics as other vessels locally and also may produce temporary obstruction by the swelling of the nearest lymph node. Thus local inflammatory edema is usually complicated by some

degree of lymphedema though this nearly always subsides with the successful treatment of the local condition.

The previous description of the effects of local incision and the promotion of tissue fluid discharge apply also to the lymphatic component of inflammatory edema since any incision is liable to open many lymph channels. The value of this type of drainage is limited by the large number of valves in the lymph system.

Summary

The function of tissue fluid is to provide a constant supply of materials to all cells and constant elimination of their waste products. The effects of stagnation of tissue fluid are discussed with special reference to anoxia. A review of the present concepts of tissue fluid formation is followed by a discussion of the three physiological changes which cause tissue fluid stagnation and edema. These are plasma protein dilution, raised intracapillary pressure, and toxic capillary dilatation with increased permeability. The application of this analysis to clinical edema is illustrated by reference to varicose veins, cardiac insufficiency, inflammation, burns, frostbite, renal diseases, toxemias of pregnancy, urticarial skin lesions and lymphedema.

REFERENCES

Anselmino, K. and Hoffman, F.: Die Ubereinstimmungen in den Klinischen Symptomen der Nephropathie und Eklampsie der Schwangeren mit den Wirkungen des Hypophysenhenlerlappenhormons, *Arch. F. Gynak.*, 147:597, 1931.

Barcroft, Joseph: Anoxemia, *Lancet*, 2:485, 1920.

Bernard, Claude: Lecons sur les Phénomènes de la vie Communs aux Animaux et aux Vegetaux, Paris, Ballière, 1878.

Chambers, R. and Zweifach, B. W.: Capillary Endothelial Cement in Relation to Permeability, *J. Cell. Comp. Physiol.*, 15:255, 1940.

Cooke, W. R.: Edema in Pregnancy.

Drinker, C. K.: The Lymphatic System, Stanford University Press, Stanford University, California, 1942, p. 66.

Drinker, C. K. and Yoffey, J. M.: Lymphatics, Lymph, and Lymphoid Tissue, ed. 1, Cambridge, Mass., Harvard University Press, 1941, Chapter 2.

Keith, N. M., Burchell, H. B., and Boggenstoss, A. H.: Electro-cardiographic Changes in Uremia Associated with a High Concentration of Serum Potassium: Report of Three Cases, *Am. Heart J.*, 27:817, 1944.

Koch, Sumner L.: The Treatment of Raw Surfaces Resulting from Burns and Wounds, *J. Iowa State Med. Society*, 32:445, 1942.

Lewis, Thomas: The Blood Vessels of the Human Skin and Their Responses, Shaw and Sons, London, 1927.

Montgomery, Mary and Montgomery, M. L.: Personal Communication.

Ogden, Eric and Sherman, R. Stanton: Physiologic Considerations in the Care of Patients with Varicose Veins, *Archives of Surgery*, 52:402, 1946.

Page, E. W. and Ogden, Eric: The Physiology of Hypertension in Eclampsia, *Am. J. Obstet. and Gyn.*, 38:230, 1939.

Schockaert, J. A. et Lambillon, J.: Un Nouveau Test Permettant de Diagnostic Précoce et Differential des États Prééclamptiques, *Brux. Médic.*, No. 41, 1937.

Starling, Ernest H.: On the Absorption of Fuids from the Connective Tissue Spaces, *J. Physiol.*, 19:312, 1895.

Trueta. J.: Treatment of War Fractures by the Closed Method, *British Med. J.*, 2:4117, 1939.

Ungley, C. C. and Blackwood, William: Peripheral Vasoneuropathy after Chilling: "Immersion Foot and Immersion Hand," *Lancet*, 2:447, 1942.

EDEMA IN PREGNANCY

WILLARD R. COOKE*

The clinically appreciable edemas which occur during pregnancy should be thought of always as falling into two groups: the insignificant and the significant, using these terms in their basic definition and not as a measure of the degree or amount of the edema. A tremendous edema may be wholly insignificant; an almost inappreciable edema may be of great significance. It is therefore necessary that the clinician have a clear knowledge of the factors which are known to be concerned in edema, and a degree of judgment adequate to the evaluation of this physical sign in each case. (Upon this basis the writer urges that the paper of Dr. Eric Ogden, immediately preceding in this Journal, be carefully read before passing on to the clinical considerations set forth herein.)

THE INSIGNIFICANT EDEMAS

1. The physiologic edema of pregnancy. This is a generalized more or less constant edema which in its minimal clinical manifestation is evident only as a tightness of the fingers and in the coarsening of the features. This type of edema has been ascribed to: an effort on the part of Nature to provide a supply of water to be drawn upon during labor and the immediate post-partum period, increased blood volume; decreased viscosity; various endocrine imbalances; capillary stasis as the result of lowered blood pressure; hypoxia; albumin-globulin shifts; inability of the kidney to eliminate sodium salts; cardiac overload; etc. None of these, except the hydremia, has been demonstrated as a constant factor. Reduction of intake of NaCl is more or less effective in some cases.

In this connection there is a widespread lack of appreciation of the real meaning of more than average or excessively rapid gains in weight during pregnancy. While the latter is more

*From the Department of Obstetrics and Gynecology, The University of Texas Medical Branch, Galveston. Received for publication September, 1946.

significant than the former, neither is *diagnostic* of actual or impending toxemia. Patients with marked losses of weight may develop a most virulent eclampsia; patients with gains of from 60 to 90 pounds may show no evidence whatever of a pathologic process. Rapid or excessive gains in weight should not be overlooked; they may be the first indication of a developing toxemia, calling for immediate intensive investigation.

2. Edema from venostasis of the lower extremities. This is a localized, intermittent edema which is nearly always unequally distributed, one leg being more swollen than the other. It almost always decreases after prolonged recumbency; often disappears entirely during the night; and often may be kept within the bounds of comfort by lying down with the legs elevated for 10 minutes or so every hour or two during the day. The degree of edema may be extreme, as in the case of a 90 pound girl whose ankles measured 42 cm. (16.5 inches) in circumference during the last weeks of pregnancy. Four factors operate directly in varying degree to cause crural congestion and phlebectasia: a. the inability of the common veins to accommodate both the external iliac return and the tremendous venous flow from the uterus via the hypogastric (internal iliac) veins; b. direct pressure of the gravid uterus upon the iliac venous system (this accounts for the usual inequality of the edema in the two legs); c. the absence of competent valves in the ovarian veins, which creates a constant hydrostatic pressure of 20–25 cm. within the pampiniform plexus during maintenance of the erect posture, thus largely preventing the utilization of these veins as compensatory relief channels for the uterine return; d. the increased intraabdominal pressure. It is probable that this type of edema (and much of the damage to the veins of the legs—to say nothing of a host of gynecologic conditions) could be prevented if women could be persuaded to maintain the horizontal position for which the genitalia and accessory structures were designed: but patients are quite unreasonable about adopting the quadrupedal position, even during pregnancy.

3. Edema incident to simple hypertension. This is very inconstant, even in severe degrees of hypertension, and be-

comes significant only when it results from hypertensive renal or cardiac damage. There are four types of simple hypertension to be taken into account during pregnancy: the familiar benign and malignant essential hypertensions; temporary and transient hypertension of psychoneurotic origin; and an unexplained type of hypertension which develops during pregnancy and disappears promptly after its termination, without demonstrable immediate or delayed side-effects. This last type may occur in one pregnancy and not in others, in a given individual.

4. Edema of apparently allergic origin is not very rare during pregnancy, and is usually transient. In one case the edema (and urticaria) was constant and became increasingly severe as pregnancy progressed, disappearing entirely within a few hours after delivery: intradermal injection of cord blood serum caused a very severe local reaction with transient generalized urticaria and mild edema.

The Significant Edemas

1. Preëclampsia-eclampsia. The edema accompanying this condition is of complex origin, especially in progressive cases. The initial factor seems to consist of an occlusive terminal endo-arteriolitis in the renal glomeruli. The cause of this arteriolitis has not been identified, although there is considerable evidence suggestive of amin-intoxication as a possibility, (especially tyramine—H. W. Johnson). The production of renin in proportion to the diminution of blood supply to the kidney probably plays a part in the increasing hypertension. With the increasing glomerular and general renal damage and dysfunction protein leakage and Na-and-K-salt and water retention occur. Both of these factors operate to increase the generalized edema. Capillary permeability is increased, especially in the cerebral area. Capillary thrombosis is usual, most commonly and markedly in the perilobular venules of the liver, where it leads to necrosis and progressive reduction of the glycogen reserve capacity.

It should always be remembered that, while edema is ordinarily our earliest and therefore most valuable warning of

eclampsia, it is neither in itself diagnostic of eclampsia nor is it a quantitative index to the severity or the rapidity of progress of the toxemia—indeed, the most highly fatal type of eclampsia is the so-called dry type, in which gross evidence of edema may be entirely lacking.

The edema of eclampsia may be lessened very markedly by a salft-free diet; but this may prove to be a psychologic boomerang, as the actual toxemia may be progressive in spite of the misleading reduction of edema. Dehydration and attempts to reduce the edema by increased protein intake are very inconstant in their effects on the reduction of edema; and neither is any more effective in the control of the actual eclampsia than the obsolete hyper-hydration and low-protein diets.

In the actual eclampsia (coma-convulsive stage) increased intracranial tension and cerebral edema have been suggested as causative of the central nervous phenomena. It seems probable that these factors may operate in the causation of coma, but not of convulsion. There is no constant relationship between the spinal or cistern pressures and the severity of the eclampsia; nor does reduction of intracranial pressure by tap or by intraduodenal instillation of magnesium sulphate have any dependable effect in controlling the convulsions. As yet, the frequent occurrence of greatly increased edema immediately after delivery and the cessation of convulsions is unexplained.

In spite of our greatly increased knowledge of the physical and physiologic pathology of eclampsia, very little of it as yet applies effectively to the practical therapy of the disease. Edema can be reduced, but little is accomplished thereby, and one must be on guard against being misled as to the progress of the actual disease. Mechanical compensation by intravenous glucose for the broken-down glycogen reserve is of very great value. As yet we have accomplished a real reduction of mortality only through the use of barbiturates and magnesium sulphate, the latter being paradoxically (on account of its lesser neuromotor-depressive effect) far superior to the former —an unexplained phenomenon.

2. Nephritis. From the practical clinical point of view (concerned primarily and almost solely with the reduction of mortality and long-range morbidity) the presence or absence of edema is somewhat immaterial in the nephritic gravida, since the general consensus of opinion today is that pregnancy should be terminated in any case of severe or progressive nephritis. This policy has reduced the maternal mortality, had little effect on the fetal mortality, and increased the life expectancy of the mother.

3. Cardiac edema also is relatively insignificant among the many criteria of cardiac incompetency in the pregnant woman. The management of these cases must be based upon intelligent coördination of both obstetric and cardiologic judgment.

4. The edema incident to varices is fully discussed by Dr. Ogden. The management of these cases should take into account the elimination of the factors due to pregnancy, after its termination.

SUMMARY

Edema is a very variable phenomenon in connection with pregnancy and its complications. It is not diagnostic of toxemia or other complicating processes, nor is its degree always a measure of the severity of the complicating disease. Extreme degrees of edema may occur in cases in which the most exhaustive studies discover no pathologic state; conversely, edema may be slight or absent in fatal cases of toxemia.

The occurrence of edema should always call for a prompt and intensive search for its cause, especially for the earliest evidences of eclampsia.

REFERENCES

Johnson, H. W.: Toxemias of Pregnancy. *Surg., Gync. & Obst.*, 70:513–516, 1940.

Ogden, Eric and Tripp, Eleanore: A Physiologic Analysis of Clinical Edema. *Texas Rep. Biol. Med.*, 5:1, 1947.

WILLIAM BUCHANAN LECTURE

ARILD E. HANSEN, Director,
The University of Texas Child Health Program*

INTRODUCTION

The idea of a University of Texas Child Health Program was conceived by the authorities of The University of Texas as a means of contributing towards improving the health of the children of this great state. In instituting the program the University authorities realized the problem was of such importance and such magnitude that it would be necessary to seek outside aid in order to support it in proportion to the extent demanded by the progressive citizens of the state. Amongst the various individuals and institutions found to be interested in aiding the University to render this important service to the state was the William Buchanan Foundation of Texarkana. The members of the Board of the Foundation adopted an extremely laudable, though a little out of the ordinary, principle of preferring not to publicize their support. There are times, however, when it is felt that distinct recognition should be given to this great pioneer of Texas who left part of his estate dedicated to the improvement of the health of its citizens. We are gathered this evening to pay tribute to William Buchanan, one of Texas' great citizens.

In developing a Child Health Program it was quite obvious that particular attention should be given to those problems which were responsible in causing the excessive number of deaths in the children of Texas. It was found that the problem of dysenteries or infantile diarrheas was of such greatness that it contributed materially to cause more children to die in Texas than in any other state of the Union. It so happens that rather recently a notable contribution has been made towards a better understanding of the actual cause of death in this condition. Evolving from these findings has been

*From the Department of Pediatrics, The University of Texas School of Medicine, Galveston. Presented October 28, 1946.

the development of a successful treatment routine for a condition in which, previously, and also in spite of excellent hospital and nursing care and wise medical supervision, it was extremely difficult to reduce the mortality rate.

There are a few things that stand out in the development of man's victory over the various diseases. Amongst these milestones in medical progress may be mentioned von Behring's development of anti-toxin in the treatment of diphtheria and the discovery of insulin by Banting and Best. Indeed there are many others, and now we have arrived at another milestone. The discovery has been made that great shifts in potassium and sodium out of and into the body cells can occur and this alteration in water and electrolyte balance results in the inability of the organism to function properly. These findings are the result of intensive work in the laboratory and clinic and demonstrations of such phenomena have been made by our speaker of the evening. The magnitude of the loss of potassium from within the cells of the body was hitherto unsuspected. Furthermore, of distinct significance is the discovery that the administration of a solution rich in potassium salts will markedly reduce the mortality from this dreaded condition.

It is with great pleasure that we present a scientist who has made important contributions in the understanding of human physiology and a clinician who has devised a system of treatment which changes a serious situation to one which can be conquered with the right means of treatment. Dr. Daniel C. Darrow is Associate Professor of Pediatrics at Yale University School of Medicine and it has been our pleasure to have him here at The University of Texas School of Medicine during the summer as Visiting Professor of Pediatrics. Dr. Darrow will discuss his experimental and clinical studies on the control of infant diarrhea. This dangerous condition has challenged the best efforts of pediatrics for years. It's successful solution by Doctor Darrow is one of the best lifesaving contributions of contemporary medicine.

ADVANCES IN THE TREATMENT OF DIARRHEA IN INFANTS*

DANIEL C. DARROW

The University of Texas Child Health Program was conceived in the broadest terms as a means of promoting the welfare of the children of Texas by research, public health activities and education of the laity as well as the medical students and physicians. The William Buchanan Foundation has supported the program because it is interested in the purposes of the program and felt that the State University was a particularly appropriate place to center these activities. Both the University and the William Buchanan Foundation have recognized that the development of a superior center for pediatric research and teaching at the medical school was one of the important phases of the program.

Throughout the country and in all fields of medicine, the type of training given by residency in well developed centers has been the most effective method of producing superior practitioners. If the men so trained are to progress with the times, the university hospitals must not only exemplify the best in medical practice, but must also have and use facilities for medical research. In pediatrics, at least the three fields of metabolic, infectious, and psychiatric diseases must be represented. A superior center for pediatrics at The University of Texas will not only improve medical practice through the men it trains, but it will, by example, lift the level of practice and education throughout the state.

Doctors Pratt, Flett and I have been permitted to take part in the research aspects as guests of the Child Health Program of The University of Texas. We have found the laboratory facilities adequate for our purposes; we have received stimu-

*From the Departments of Pediatrics, Yale University School of Medicine and The University of Texas School of Medicine. Presented October 28, 1946, in connection with the Pediatric Postgraduate Conference conducted under the auspices of The University of Texas Child Health Program in coöperation with the Maternal and Child Health Division of the Texas State Board of Health.

lation and coöperation from the investigators on the faculty; the people of Texas, as exemplified by Bradford Home in Dallas, are ready to support research in medicine. My colleagues and I have had the opportunity to help in the training of the residents in Galveston and Dallas. We found the men were intelligent and willing to learn and they deserve the best that Texas can give. Despite the set-backs of the war, the Texas Child Health Program has done much toward developing a superior center for research and resident training at the medical school and continued support can bring this phase to fruition.

Tonight I shall talk of the advances in the treatment of diarrhea. These advances are largely the result of work made possible in other medical schools by establishment of pediatric centers such as is proposed by The University of Texas Child Health Program. Back of this progress you will see laboratory observations taken to the bedside and bedside problems taken to the laboratory and most of the work done by men engaged primarily in the study of disease rather than preoccupied with the application of current medical knowledge.

Although considerable progress has been made, diarrhea diseases are still among the chief causes of death in the first year of life. In 1944, the State of Texas reported 1372 deaths from diarrhea in infants under one year of age, while all the United States in the same year reported only 1361 deaths from poliomyelitis for all ages. Texas reported over twice as many deaths from diarrhea as the highest number in any other state. The number of deaths per thousand live births was 8.3 in Texas and this rate was exceeded only by the rates of New Mexico and Arizona (15.5 and 12.6 respectively). On the other hand, the best record, that of Oregon, is only 0.7 per thousand live births and with the exception of Maine, Ohio, and many Southern states, there are no rates over 3. In New Mexico and Arizona the deaths for "non-whites" are almost 1 of 30 live births. In Texas the death rates for "non-whites" is lower than for whites. In Mississippi the rate for "non-whites" is 3.6 and almost as low as for the state as a whole. The highest death rates in the Southwest are almost

certainly confined largely to the Mexican and Indian populations which in Texas are hidden by recording only Negroes as "non-whites," while in Arizona and New Mexico the problem comes into view by classification of the Indians and Mexicans as "non-whites." The death rate of certain parts of the Indian and Mexican populations of Texas is almost certainly as high as that of Arizona and New Mexico.

The decreased incidence of diarrhea in many parts of the country is not altogether explained. Diarrhea in babies decreases with better sanitation since dysentery accounts for a large number of cases and where adults suffer frequently from dysentery, babies show a high incidence of Shigella infections. Diarrhea decreases when public sanitary facilities are adequate. Deaths from diarrhea occur almost exclusively in the first two years of life and chiefly in the newborn period and in undernourished babies. Measures which decrease the incidence of undernutrition and improve the care of the newborn decrease the deaths from diarrhea. Finally, deaths occur in cases where, through ignorance on the part of the parents or the doctors, proper treatment is not instituted. The solution of Texas' problem of infantile diarrhea will demand improved public sanitation and better education of the public. However, in the final analysis these factors alone will not take care of individual cases unless the pediatricians in particular and the physicians in general are acquainted with the best methods for treating the sick baby.

In the individual case, the treatment seeks to prevent severe dehydration or to overcome dehydration if it has already developed. Without the development of dehydration, practically no deaths occur from diarrhea. With proper replacement of the losses of water and electrolyte, the deaths can be reduced to a very low figure. The treatment demands a considerable attention to details and involves several important procedures no one of which is effective alone. In describing the treatment I shall try to illustrate the observational background that demonstrated the usefulness of the particular phase of the treatment.

It is likely that early attempts to treat diarrhea involved changes in the diet which sought to decrease the number and volume of the stools. This search for a food which will not aggravate the diarrhea led to the substitution of gruel for milk, the use of buttermilk and protein milk. However, Marfan popularized the only procedure that has stood the test of time; this is the administration of water alone as long as the stools are copious and watery and as long as evidences of so-called intestinal intoxication persist. He wrote his paper about 1890 but gives credit to Luton of Rheims who used it in 1874. Small amounts of salt and glucose can be added to water given orally without aggravating a severe diarrhea, but until considerable recovery has taken place, milk in particular aggravates a watery diarrhea. In many clinics nothing is given by mouth since adequate fluids can so easily be given parenterally. However, except when there is vomiting, clear fluids by mouth do not aggravate a diarrhea and can be given after partial replacement of the deficit of water and electrolyte. Thus the first phase of treatment of diarrhea is withholding food until the stools are no longer watery, all evidences of dehydration are overcome and considerable recovery has taken place. Replacement of water and electrolyte can be accomplished by the oral route in mild cases where there is no vomiting. The oral replacement of water and electrolyte usually requires withholding milk for 12 to 24 hours. The cases with marked dehydration require parenteral fluid therapy before the oral administration of fluids is feasible. In the most advanced cases starvation may have to be carried out frequently for four or sometimes for ten days.

The actual mechanism by which food aggravates the symptoms of diarrhea have not been discovered and described, but the clinical facts are clear-cut and were best described by Finkelstein, Myer and coadjutors. These observations led Finkelstein to call the group of symptoms alimentary intoxication. The essential findings are as follows: Certain babies react to food with fever, loss of weight, listlessness, pallor, and evidences of poor circulation. Usually diarrhea develops or is aggravated, but loss of weight may occur without diarrhea.

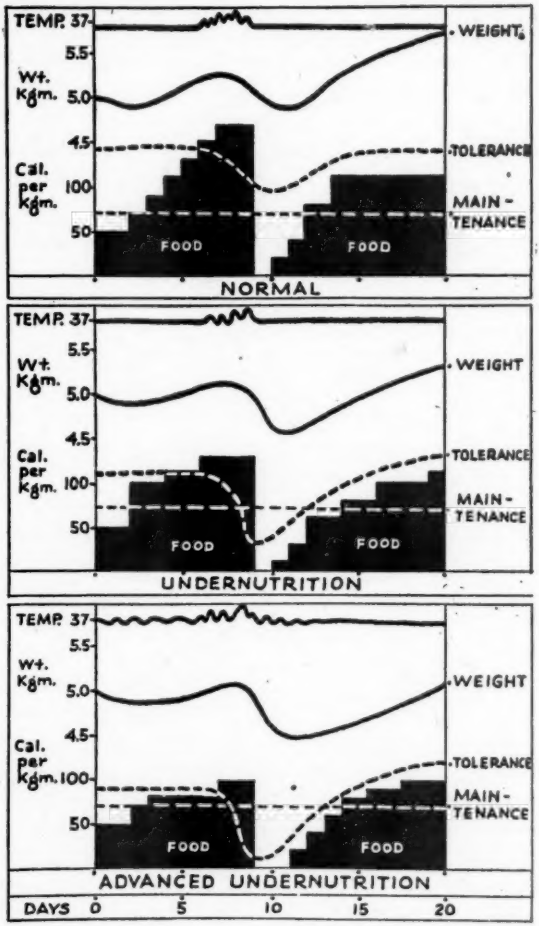


Chart 1 is a diagrammatic representation of the reaction of normal and undernourished babies to increased feedings. The line labelled "Maintenance" represents the food intake which will lead to no gain or loss of weight; the line labelled "Tolerance" represents the amount of food which leads to symptoms. Note that tolerance is lower in undernourished than in normal infants and that tolerance is decreased by amounts of food that lead to symptoms.

Vomiting eventually occurs. These phenomena can be illustrated in almost any patient with diarrhea and in many undernourished babies and some babies suffering from infections. Pirquet and others spoke of the phenomena in terms of food tolerance. I shall illustrate the phenomena by diagrams.

The first diagram shows that as food is increased the rate of weight increase slows up when a certain intake of food is exceeded. If food is further increased, fever develops and a loss of weight occurs. Vomiting and diarrhea may also develop. The amount of food which produces these symptoms is said to exceed the tolerance. In an undernourished baby the same phenomena are more easily elicited and the tolerance is exceeded at a lower level of intake. In the advanced stage of undernutrition the baby may not be able to take sufficient food to meet his caloric requirements. This state was called by Finkelstein, "decomposition." Usually evidences of dehydration are obvious in such babies. A baby who has suffered or is suffering from diarrhea illustrates these phenomena very readily; he acts like a baby with moderate or advanced undernutrition even though he may look well nourished. A period of fasting will increase the tolerance of a baby suffering from diarrhea; restoration of tissue water and electrolyte will also increase the tolerance. However, if the diarrhea continues to be watery and copious, food will always aggravate the diarrhea and may induce fever, vomiting and collapse. It requires considerable clinical experience always to judge when food will be tolerated, but cautious additions of food in small amounts enable a physician to institute feedings without danger if the course of the illness is carefully followed. Diarrhea usually requires withholding food for 24 hours and may require a period of fasting of three to four days and recovery has occurred after eleven days of starvation.

The applications of these observations are relatively simple. First food must not be given at a time when watery diarrhea, dehydration or fever indicate that aggravation of these symptoms is likely to develop. Usually small amounts of food are tolerated at first and then as recovery occurs more food can be taken. The food can safely be diluted so as to cover the

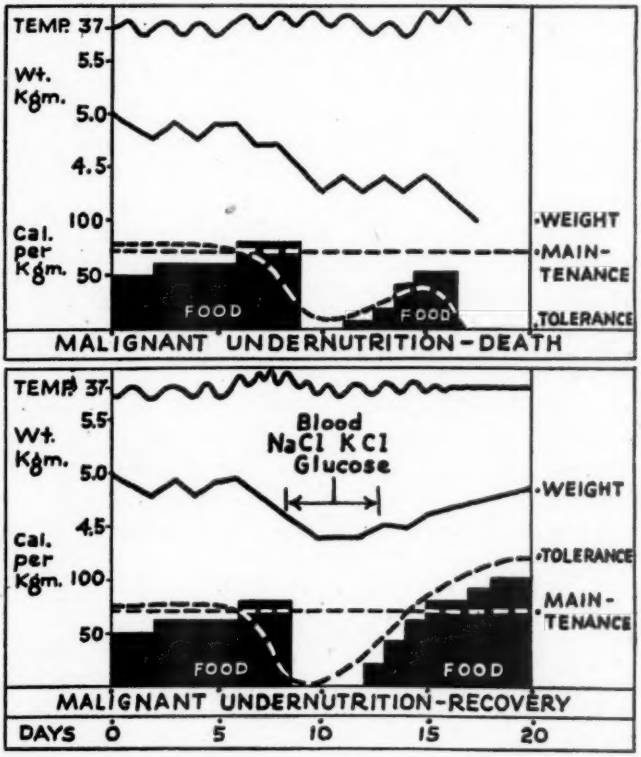


Chart 2 represents the same type of reactions as chart 1, but in malignant undernutrition ("decomposition" of Finkelstein). By definition, these babies are unable to take enough food to grow without developing symptoms of alimentary intoxication. Since deficit of water and electrolyte plays a role in the pathogenesis, treatment with blood, salt, and glucose solutions leads to recovery in some cases.

water requirement—usually about 150 cc. per kilogram per day in babies suffering from diarrhea. The initial food should give about 10 to 20 calories per kilogram per day and the daily increases should be from 10 to 20 per day until an intake of 90 to 100 is given. This usually suffices for growth. More may be given if a trial of several days shows that more is necessary and will be tolerated. If the food aggravates the diarrhea, a period of fasting may again be necessary and gradual initiation of feedings started when improvement has taken place.

There are some indications that inability to maintain fluid and electrolyte balance is one of the causes of intolerance to large amounts of food. Banning found that many premature infants who fail to thrive on whole milk mixtures have acidosis. Martins da Silva, Stevenson and I showed that premature infants retain chloride without sodium when fed protein milk powder. This leads to marked acidosis and the appearance of dehydration without the development of diarrhea. In Galveston a similar imbalance of electrolyte retention was shown in babies recovering from diarrhea, who were fed milk mixtures before the stools were satisfactory. These findings suggest that the imbalance of electrolyte may be one of the features of food intolerance. Detailed study of the phenomena will be required to establish this assumption.

Until the work of Brennemann showed that modification of milk protein is the most important change necessary to make cow's milk suitable for infants, pediatricians tried many mixtures. Modifications of the proteins can be brought about by boiling or cooking the milk, by souring, by coagulation with rennet as in Finkelstein's protein milk or by a number of processes by which milk is powdered or concentrated. Such modifications are so prevalent today that their importance may be forgotten.

Experiments in Germany by Steinitz, Meyer and others showed that high fat diets are more likely to lead to loose stools than milks somewhat low in fats. A suitable concentration of fat can be obtained by using buttermilk, skimmed milk or a mixture of equal parts of boiled skimmed and whole milk.

The evidence is now clear that carbohydrates do not aggravate a diarrhea and babies do better when carbohydrate, equal to 5 to 10 per cent of the milk, is added to the mixture. Babies who are ready for food can take any of the relatively simple mixtures, and it is futile to search for a peculiar mixture suitable for a particular baby suffering from diarrhea.

The explanation of the weight loss received its first outstanding study in Dorpat, Esthonia. About a hundred years ago, Carl Schmidt published his studies on cholera and dysentery. He appreciated that the body fluids were of different composition outside and inside of the cells. His terminology suggests his comprehension of the problem, since he spoke of "Capillartransudat," by which he referred to what we would call interstitial fluid; he spoke of the stools as "Darmcapillartransudat." He published his data in the contemporary system of oxides of the various cations, but calculated the equivalence of the various elements. He clearly showed that diarrhea leads to large losses of water, sodium, chloride, and potassium. His work was done fifty years before the theory of electrolyte dissociation was developed by Arrhenius. Doubtless his work would have been better understood if he had presented his tables in equivalents and if the science of physiology had been prepared for his advanced views.

Shortly after 1890 the establishment of separate professorships of pediatrics together with hospitals and laboratories for the study of diseases of children led to the development of the great school of pediatrics in Germany. German pediatricians made many attempts to solve the problem of dehydration in diarrhea. They appreciated that the decrease in body weight was due to loss of water and that electrolyte was excreted along with the water. Steinitz showed that the acidosis in diarrhea was due to loss of sodium and potassium. Tobler measured the losses of electrolyte and analyzed tissues. He showed that the tissues lost more chloride and potassium relative to nitrogen than would be the case if tissue fluids as a whole were lost. He showed that the muscles of babies dying of diarrhea contained less than the normal proportion of

potassium. The losses of weight were accounted for chiefly by losses of water from subcutaneous tissues and muscle.

In spite of this knowledge but few attempts were made in Germany to replace the lost electrolyte. This failure was apparently dependent, first, on the idea that the loss of potassium represented a disintegration of the cells as a whole and, second, on the teaching that while administration of physiological saline produced immediate improvement, the condition became worse if the watery stools continued. The Germans sought to change the character of the stools by a period of fasting and giving a food which changed the stools from watery ones containing large amounts of sodium and potassium to ones containing large amounts of calcium and magnesium soaps. In this they were partially successful by the use of protein milk, buttermilk and other mixtures. The German pediatricians observed temporary improvement following the administration of salt solution, but felt that, unless the character of the stools changed, a relapse into a worse state would occur.

Although American pediatrics was founded by men trained in Germany, American pediatricians followed different lines of investigation and explored the possibilities of replacement therapy more carefully. In this they followed the successful modification of cholera in England by administration of sodium chloride. The development of micromethods for blood analyses enabled American investigators to follow the changes in composition of the plasma quite accurately. The demonstration of acidosis with lowering of the serum bicarbonate concentration by Howland and Marriott and Schloss and Stetson was accompanied by proof that sodium bicarbonate restored serum bicarbonate concentrations. However, both groups of workers stated that the immediate improvement was not followed by a lowering of the mortality rate. Blackfan and Maxy observed a gratifying decrease in mortality following the use of adequate amounts of physiological saline by intraperitoneal injection, but a better quantitative approach was needed to exploit the possibility of this therapy.

Hoag and Marples; Hartman; Hamilton, Kajdi and Meeker; Karelitz and Schiff showed the nature of the disturbances in

electrolyte concentration of serum. Various combinations of sodium chloride, sodium bicarbonate or sodium lactate were used to overcome water and electrolyte deficits. These studies often used restoration of serum electrolyte concentration as evidence of successful treatment when observation of the patients indicated that the patients were not restored to normal when serum concentrations were normal. The treatment was dominated by the concepts of Gamble who divided body fluids into intracellular and extracellular fluids. Only the extracellular disturbances were considered accessible to treatment, since the membranes were thought to be practically impermeable to cations and sodium was thought to be excluded from the cells.

Holt, Courtney and Fales studied the excreta and realized that potassium was being lost as well as sodium and chloride. Holt, Jr., informs me that his father consulted Jacques Loeb as to possible solutions for restoring the deficits of potassium. Apparently some form of Ringer's solution was recommended and perhaps tried. However, doctors were so conscious of the possibility of producing heart block by too high a concentration of potassium in serum that adequate amounts of potassium were never given.

Gamble, Butler, and McKhann demonstrated by balances that diarrhea leads to loss of intracellular fluids with potassium and very little nitrogen proportionately. They did not attempt to restore potassium for the reasons that appear in the following quotation:

"The presence of an intracellular fluid loss complicates our conception of the process of dehydration and disturbs our confidence in the therapeutic adequacy of parenteral treatment. Since repair solutions must be placed in the vascular or in the interstitial compartment, they cannot contain with safety the intracellular materials, such as potassium and phosphate, at concentrations above the small values prescribed for them in extracellular fluids. The solution devised by Hartmann contains potassium appropriately to this extent. It is evident, however, that such solutions cannot provide an adequate replenishment of intracellular materials. Parenteral therapy has a large, often dramatic, effectiveness. It must be admitted, however, that it is not always successful and it may be hoped

that recognition of an additional pathologic process in the situation will eventually produce supplementary measures which will provide a more complete control of the severe stage of diarrheal disease."

From the above it is obvious that potassium would not be tried as long as cell membranes were considered to be practically impermeable to potassium. Investigators had to keep in mind that a rise in concentration of serum potassium to a little over twice the value found normally in serum would produce heart block. A new concept of the relationship between intracellular and extracellular fluids had to be developed by new methods of observation. The analysis of tissues was the new method of observation and was undertaken by a number of investigators, particularly Hastings and Eichelberger in Chicago, Fenn and others in Rochester and Yannet, Harrison, Miller and myself in New Haven. This work went on independently and along similar lines and showed that cells contained moderate amounts of sodium and variable amounts of potassium. However, we were hardly prepared for the finding that about half the potassium of muscles may be replaced by sodium following a diet low in potassium. Hepel first described these facts, but Miller and I made the same observation independently at about the same time. We were thus confronted with the fact that the muscles may show a wide variation in composition and sodium and potassium readily cross the cell membranes. The distribution of electrolyte in the body is apparently not dependent on a peculiar property of the membranes which excludes sodium from the cells and confines potassium to the cells. Within the cells potassium must form complexes which lead to a higher concentration of potassium than sodium, but if potassium is lost, sodium tends to replace potassium in about equivalent amounts. These observations have been supplemented and confirmed by the use of radio-active isotopes. The evidence indicates that radio-active sodium comes into equilibrium with intracellular sodium more rapidly than radio-active potassium comes into equilibrium with intracellular potassium. The cell membranes are, therefore, for all practical purposes, freely permeable to

univalent cations and the function of the membranes is to confine the cytoplasm and not to exclude the sodium or confine the potassium.

In the light of these new concepts of the function of cellular membranes, the medical and physiological problem was to discover the variations in tissue composition and their effect on acid-base equilibrium in the serum and the changes in functions of the cells which accompany changes in body electrolyte. Replacement of muscle potassium by sodium has been shown to occur in response to diets low in potassium, following parenteral treatment with solutions containing only sodium chloride and glucose, following administration of desoxycorticosterone acetate and certain steroids found in the adrenal glands, in alkalosis and probably other conditions.

The general implication of the lability of tissue composition is illustrated in tables 1 to 4. The concentrations in intracellular and extracellular fluids are represented together with the total amounts. The composition of extracellular fluids is given as the ultrafiltrate of plasma, while the composition of intracellular fluids is represented by intracellular fluid of muscle. Muscle comprises about 70 per cent of the total intracellular fluid and is sufficiently characteristic to represent all intracellular fluids. The tables illustrate certain changes in composition which have been shown to occur in experimental animals and patients.

Table 1 illustrates the composition of the body of a ten-kilogram baby. The values for intracellular sodium and potassium are high normal values, but in essentially normal animals intracellular sodium can almost disappear and intracellular potassium can decrease about ten per cent. Attention should be directed to the fact that intracellular sodium is almost one-sixth of normal extracellular sodium or two-thirds of extracellular bicarbonate. These variations must be considered in evaluating acid-base equilibrium of serum. In one type of acidosis, da Silva, Stevenson and I found practically all the intracellular sodium transferred to extracellular fluids, at a time when there was no evidence of loss or gain in potassium.

TABLE 1

Normal Baby

		Concentration per kgm. of water		Total Amount	
		Extra-cellular	Intra-cellular	Extra-cellular	Intra-cellular
HCO_3	mM.	28	10	70	45
Cl	mM.	111	3	277	15
Na	mM.	145	12	362	54
K	mM.	3	157	8	706
P	mM.	2	101	9	455
Solids	gm.		306		1377
N	gm.		49		222
H_2O	kgm.			2.5	4.5

In this and the subsequent tables, the contents are based on the solids of a 10 kgm. baby including the solids of bones and connective tissue, which are not shown in the tables. The concentrations of extracellular fluids are represented by those of an ultrafiltrate of serum, while the intracellular concentrations are represented by those of the intracellular phase of muscle. Total amounts of each electrolyte equal the concentrations multiplied by the respective volumes of water.

Table 2 shows the effect of loss of extracellular electrolyte without appreciable change in body water. In this case almost no shift in electrolyte occurred, although there is a marked decrease in extracellular water with a corresponding increase in intracellular water. Similar changes probably take place in patients.

Tables 3 and 4 show two types of disturbances in body water and electrolyte in which deficit of potassium is a prominent feature. Alkalosis produced experimentally by deficit of body chloride induces excessive retention of sodium in the cells associated with equivalent deficits of potassium (see table 3). Thus alkalosis per se seems to alter the composition of muscle. On the other hand, Powers and Kepler have shown that certain cases of Cushing's syndrome have alkalosis associated with deficit of muscle potassium. Experimentally I have shown that the same association of alkalosis with deficit

of potassium can be produced in animals by repeated injections of desoxycorticosterone acetate. Thus deficit of potassium apparently tends to lead to serum alkalosis when renal function is relatively normal. Alkalosis does not usually develop when potassium deficit is produced by diets low in potassium. Moreover, the studies on diarrhea show that deficit of potassium may develop in the presence of acidosis and indeed in some cases apparently lead to acidosis owing, in part, to transfer of sodium to the cells (see table 4). In some cases of diarrhea no evidence of increase in intracellular sodium accompanies loss of body potassium. The point to be emphasized is that disturbances in body water and electrolyte are now known to be accompanied by shifts of both water and electrolyte between extracellular and intracellular fluids. Furthermore, methods of study have been developed which bring these shifts into view.

Keeping in mind the concept of the lability of tissue water and electrolyte, studies were undertaken which demonstrate the changes in intracellular as well as extracellular electrolyte

TABLE 2

Baby with Deficit of Extracellular Electrolyte

		Concentration per kgm. of water		Total Amount	
		Extracellular	Intracellular	Extracellular	Intracellular
HCO_3	mM.	24	10	50	49
Cl	mM.	101	3.3	210	16
Na	mM.	131	9	275	44
K	mM.	3.3	143	7	706
P	mM.	2	92	6	455
Solids	gm.		280		1377
N	gm.		45		222
H_2O				2.07	4.93

The table represents deficit of extracellular electrolyte without change in total body water. The deficits are Cl, 66 mM., and sodium, 97 mM.

TABLE 3

Baby with Alkalosis and K Deficit

		Concentration per kgm. of water		Total Amount	
		Extra-cellular	Intra-cellular	Extra-cellular	Intra-cellular
HCO_3	mM.	34	10	85	42
Cl	mM.	105	2.2	262	9
Na	mM.	145	55	362	231
K	mM.	2.2	112	6	470
P	mM.	2	108	8	455
Solids	gm.		328		1377
N	gm.		53		222
H_2O	kgm.			2.5	4.2

The table represents the conditions produced in experimental animals by diets low in potassium or repeated injections of desoxycorticosterone acetate. The deficits are Cl, 21 mM.; potassium, 238 mM.; and water, 0.3 kgm. There is an excess of 177 mM. of sodium which is accounted for by sodium which has replaced the deficit of intracellular potassium.

TABLE 4

Baby with Deficit of Sodium, Chloride and Potassium

		Concentration per kgm. of water		Total Amount	
		Extra-cellular	Intra-cellular	Extra-cellular	Intra-cellular
HCO_3	mM.	10	10	18	37
Cl	mM.	105	5	185	18
Na	mM.	130	22	229	80
K	mM.	5	129	9	476
P	mM.	2	123	4	455
Solids	gm.		373		1377
N	gm.		60		222
H_2O	kgm.			1.76	3.70

The table represents one of the conditions produced by diarrhea. There is acidosis with low concentration of serum chloride and deficit of potassium with partial replacement of potassium by sodium within the cells. The deficits are Cl, 89 mM.; Na, 107 mM.; K, 229 mM.; and water, 1.54 kgm.

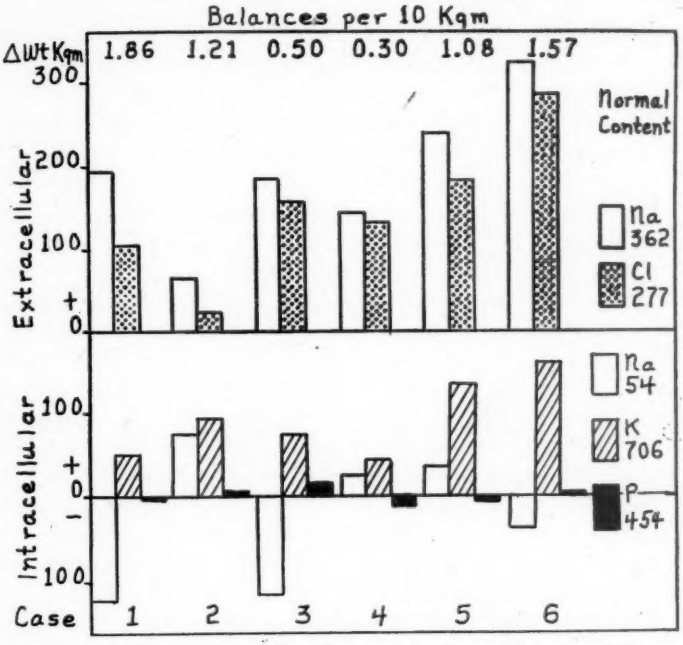


Chart 3 shows a summary of the results reported in the Journal of Pediatrics, 28:515, 1946. The reader is referred to this article for the details, particularly the method of calculating extracellular and intracellular retentions. The balances represent the retentions after recovery from severe dehydration, acidosis and diarrhea. The duration of the balances is 6.7, 9.08, 4.6, 9, 13.04, and 10.37 days respectively. Using the final weights, all data were recalculated to amounts for 10 kilogram of tissue. The figures on the right represent estimated normal contents. The retentions of extracellular electrolyte are probably somewhat large, owing to inability to estimate losses in sweat; the balances in intracellular electrolyte are probably fairly accurate. The positive values indicate deficits during dehydration and the negative values for intracellular sodium indicate excessive contents of sodium within the cells during dehydration.

in diarrhea. The data collected were balances of nitrogen, water and electrolyte during recovery together with serum concentrations. By assuming that chloride remains exclusively extracellular, the serum concentrations and balances of chloride measure the volume of extracellular fluids. From this measurement, the corresponding changes in extracellular sodium are estimated, and thereby the changes in cellular composition.

Chart 3 represents the findings in six cases of severe diarrhea. Since the babies were relatively normal at the end of the period of observation, the water and electrolyte retained measures the deficit at the beginning of the study. In other words, the retentions are essentially the losses that produced the dehydration. The chart shows that large amounts of potassium as well as sodium and chloride were retained in all cases. In the last two cases the retention of potassium is almost one-fourth of the estimated total normal content. Since in cases 1 and 3 considerable sodium left the cells during treatment, there was an excess of intracellular sodium when the babies were dehydrated. This excess of intracellular sodium probably entered the cells owing to deficit of potassium. In case 1 the patient had a very low serum bicarbonate and retained fairly large amounts of chloride and but little sodium. Hence his acidosis was dependent on the transfer of sodium to the cells rather than loss of sodium from the body as a whole. To a less striking degree the same findings are shown in case 3. The deficits of potassium are not accounted for by loss of nitrogen nor are they accompanied by intracellular deficit of phosphorus. The findings demonstrate a new mechanism for acidosis and bring into view the magnitude and significance of the losses of potassium. Data obtained in Galveston show that transfer of sodium to the cells occurs when no potassium is administered in treatment of diarrhea. The studies show that potassium chloride as well as sodium chloride, sodium bicarbonate and glucose solutions are indicated in the treatment of dehydration due to diarrhea.

The method of treating diarrhea with potassium as well as sodium salts was worked out and tested in coöperation with Clifton Govan, a native Texan. The only change from previous

treatment was replacement of body electrolyte by a solution having the composition shown in table 5. This solution contains the equivalent of somewhat more bicarbonate than serum, somewhat less sodium and about the same amount of chloride as serum. The potassium concentration is 35 mM. per liter, almost ten times the concentration of serum and three times the concentration leading to heart block. During the epidemic in Baltimore 17 of 59 cases died before potassium therapy was tried, while with the addition of potassium only 3 of 52 died. All who observed the patients were impressed that these babies looked better than one was accustomed to observe under the older treatment. The studies we have carried on in Galveston and Dallas as a special project of The University of Texas Child Health Program and because of the support of the William Buchanan Foundation are not finished, but should supplement this information. The mortality record in Texas is equally low.

TABLE 5

Composition of Solution per Liter

	Gm.	mM.
$NaHCO_3$	4.4	52
KCl	2.7	36
NaCl	3.0	69

Owing to loss of sweat during the balance determinations, the apparent retentions of sodium and chloride are probably somewhat larger than the deficits of these ions at the beginning of the observations of table 1. From animal experiments I estimate that the deficit of sodium and chloride in severe dehydration is equivalent to from one-third to one-half of the normal extracellular electrolyte. The deficit of potassium may be from 10 to 25 per cent of the estimated normal content. The potassium deficit probably cannot be replaced entirely by parenteral means, but the solution which Govan and I devised can be used without undue danger. In severe dehydration the estimated therapeutic dose of the solution during the first 24 hours is 80 to 100 cc. per kilogram. This should be

given after the circulation has improved following the intravenous injection of saline solutions and glucose. It must be given over a period of four hours so that there is no danger of a rise in the concentration of potassium in serum to toxic levels. If the solution is given by continuous intravenous drip, it should be diluted about one part to 2 parts of 5 or 10 per cent glucose. Because of the possibility of undetected increase in the rate of intravenous injections, I always give the potassium solutions by hypodermoclysis.

The intake of water without salt should be kept high by the use of intravenous solutions of glucose. Usually the total water intake should be between 150 and 200 cc. per kilogram and of this total one-half to two-thirds should be given as 5 to 10 per cent glucose. It is important that the glucose solution be made up in water and not salt solution. We have evidence in one case that potassium did not enter the cells when the concentration of sodium in serum remained high. Administration of sufficient glucose solution in water should overcome this difficulty. It should be remembered that water without salt is needed to cover imperceptible loss and the water necessary for urine formation.

After some recovery has taken place, any further requirement of electrolyte and water can usually be given orally by offering 150 to 200 cc. per kilogram of body weight of a solution containing one part of the potassium containing salt diluted with two parts of 5 per cent glucose in water.

The potassium solution contains the equivalent of about 50 mM. of sodium bicarbonate per liter. The administration of the solution as outlined above led to restoration of serum bicarbonate to approximately normal figures within 24 hours in practically all the cases studied in Texas. It will probably seldom be necessary to administer additional sodium bicarbonate or sodium lactate when this solution is used.

The treatment of shock is one of the most important phases of therapy in severe diarrhea. The term shock has been used for a number of conditions of dissimilar origin. I refer to the changes taking place in the cells as a result of the prolonged anoxia accompanying the loss of electrolyte in diarrhea. Long

and others showed that the liver in particular undergoes changes in its enzymes as a result of the slow circulation in hemorrhagic shock. The peculiar susceptibility of the liver to injury from slow circulation is explained by the fact that it gets about 70 per cent of its oxygen from venous blood. If this type of anoxia is permitted to persist, the loss of enzyme activity cannot be restored. Similar changes probably take place in other tissues—perhaps with the production of toxic by-products. The only known method of reversing the process is improvement of the circulation. The work of Winkler and others shows that in the shock due to electrolyte deficit, salt solutions are the most important means of improving the circulation. However, salt therapy is made considerably more effective by transfusions of blood and, to a lesser extent, plasma. Practically all cases of severe diarrhea should be treated for shock immediately as an emergency in which delay for a few hours may produce irreversible changes. Since it is impossible to diagnose shock by clinical means alone, a physician should treat as shock many cases in which the subsequent course would show that the treatment was not necessary. It is for this reason that I would advise intravenous salt solution or a balanced solution containing sodium bicarbonate or lactate as well as sodium chloride (20 to 30 cc. per kilogram) diluted with a little glucose in severe cases. In addition, plasma or blood should also be given (20 to 30 cc. per kilogram). The concentrated state of the blood is not a contra-indication to transfusions. The treatment is also important as a preliminary to the administration of the potassium solution, since it assures better circulation and overcomes oliguria and diminishes the dangers of potassium intoxication.

Powers introduced the use of transfusions in diarrhea and correctly stated that the treatment was for shock and not for anemia. His comprehensive plan for the treatment of diarrhea apparently produced as good results in 1926 as have been reported in any of the more recent modifications before the use of potassium was tried.

Rapaport and Dodd have observed certain deficiencies appear after the initial deficit of water and sodium chloride has

been overcome. Robinson noted strikingly low concentrations of potassium in serium (below 1 mM. per liter). He thought a fatal outcome could be predicted if the serum potassium became low and failed to return to normal. Rapaport and Dodd have found low concentrations of potassium phosphate and calcium during convalescence. Certain patients looked pale, became irritable and showed increased muscle tone. Although the signs of tetany were usually absent, these patients were favorably influenced by intravenous calcium and had low concentrations of calcium in the serum. One of the babies in Galveston had a convulsion about eight hours after initiation of treatment and the next morning, about four hours after the convulsion, the serum calcium was somewhat low (8.5 mg. per 100 cc.) and a Chvostek sign was present. None of the other patients studied in Texas have shown low serum calcium or signs referable to tetany. However, the observations of Rapaport and Dodd indicate that intravenous injections of calcium gluconate may be indicated. Although we have observed low serum potassium following therapy with sodium salts alone, serum potassium remains normal or slightly high when the solutions containing potassium are used. Although it is known that magnesium is lost in diarrhea, data are not available to prove that this ion should be given. Thus the treatment may alter the equilibrium so that deficiencies appear which would not otherwise be an important phase of diarrhea.

Although parenteral infections may play a role in precipitating an attack of diarrhea, I am more and more impressed with how seldom the outcome in cases of diarrhea is favorably influenced by local or general therapy aimed at overcoming the parenteral infection. Mastoidotomy has seemed to increase the mortality in my experience. This is not surprising when it is recalled that all operations are hazardous in the presence of dehydration. Diarrhea alone is not an indication for mastoidotomy or myrengotomy, although these operations may be undertaken if the same criteria are demanded for the diagnosis as in other conditions. Medical treatment with peni-

cillin is probably without danger, but oliguria makes the use of sulfa drugs somewhat hazardous.

In Shigella infections, sulfadiazine is probably more effective than the relatively insoluble drugs. In infants, sulfadiazine may be given if it is realized that it will only reduce the period during which organisms will be recovered from the stools, but will not shorten the period of diarrhea sufficiently to permit curtailment of water and electrolyte therapy. Sulfadiazine has not clearly lowered the mortality in Shigella infections of infants.

Our data clearly show that nitrogen is readily replaced as soon as food is taken. Hence parenteral amino acids are not indicated unless an unusually prolonged period of fasting is necessary.

Methemoglobinemia has been observed in diarrhea in adults, but has not been considered a part of the picture in babies. Comly observed several babies with the presenting symptom of cyanosis which he showed was due to methemoglobin. Comly and others have shown that this type of methemoglobinemia is associated with administration of milk mixtures made up with well water containing high concentrations of nitrates. In some cases diarrhea accompanied the cyanosis. Ingestion of water with high nitrates produced methemoglobin in young babies, but not in adults and children. Large doses of bismuth subnitrate have been shown to produce methemoglobin. It is likely that young babies get methemoglobin either from an unusual tendency to absorb nitrite or from an unusual tendency to reduce nitrates to nitrites.

In Galveston we observed two babies with the presenting symptom of severe diarrhea with dehydration and marked acidosis. In addition there was conspicuous cyanosis at a time when there was no pulmonary change and the circulatory disturbances were no greater than in many dehydrated infants. One of the babies had convulsions presumably dependent on anoxia. Doctors Ormsby and Pratt identified methemoglobin in one of these cases. Methylene blue was given to both cases with apparent benefit, but measurements

of an immediate change in methemoglobin concentration were not made.

In retrospect I believe I have seen similar cyanosis in a few cases of diarrhea shortly before death. Indeed, Marriott described black, thick blood as characteristic of advanced dehydration and it was blood answering this description that led to closer observation of the peculiar cyanosis of these babies. One of the babies came from Freeport and may have received well water, but the other came from Galveston and received water from the city supply. The susceptibility of infants to methemoglobin should be explored not only with respect to intake of nitrates, but also the changes in intestinal flora, the intestinal absorption of nitrites and the possibility of changes in other oxidation and reduction systems to which infants' hemoglobin is exposed. I believe more cases of methemoglobinemia will be found in advanced cases of dehydration, now that the electrolyte disturbances can be more successfully treated.

Dr. Pratt observed two deaths from apparent respiratory failure in the only fatal cases given the new treatment in Galveston and Dallas. The deaths were not accompanied by abnormal concentration of calcium or potassium in the serum, the diarrhea had stopped and the infants were thought to be recovering. We have not previously seen similar deaths in diarrhea. We can only speculate that new defects will appear as we are more successful in controlling deficits of sodium, chloride and potassium.

I would, therefore, close with the idea that a new era will probably be introduced in the study of diarrhea. We want to know how dehydration and electrolyte disturbances affect the functions of the cells. The explanation of death is probably not purely circulatory or due to changes in electrolyte concentrations. Within the cells, the complex metabolic process cannot be carried on if circulatory or electrolyte changes alter the enzyme systems. The production of methemoglobin may be due to absorption of nitrites or some oxidative agent; it may be the result of disturbances produced within the body as a whole. Methemoglobin is an enzyme which can be altered

by diarrhea. I believe we should now seek information as to the effect of changes in water and electrolyte on cellular enzymes.

BIBLIOGRAPHY

Butler, A. M., McKhann, C. F. and Gamble, J. L.: Intracellular fluid loss in diarrhea disease. J. Pediat., 3:84, 1933.

Comly, H. H.: Cyanosis from nitrates in well water. J.A.M.A., 129:112, 1945.

Darrow, D. C. and Yannet, H.: The changes in distribution of body water accompanying increase and decrease in extracellular electrolyte. J. Clin. Invest., 14:266, 1935.

Darrow, D. C.: Tissue water and electrolyte. Ann. Review of Physiol., 6:95, 1944.

Darrow, D. C.: Relation of tissue composition to problems of water and electrolyte balance. New Eng. J. Med., 233:91, 1945.

Darrow, D. C., Martins da Silva, M. and Stevenson, S.S.: Production of Acidosis by Protein Milk. J. Pediat., 27:43, 1945.

Darrow, D. C.: The retention of electrolyte during recovery from severe dehydration due to diarrhea. J. Pediat., 28:515, 1946.

Darrow, D. C.: Treatment of diarrhea in infants. Interne., 12:594, 1946.

Department of Commerce, Bureau of Census, Vital Statistics. Special Reports, State Summaries. Volume 24.

Faucett, R. L. and Miller, H. C.: Methemoglobinemia occurring in infants fed milk diluted with well water of high nitrate content. J. Pediat., 29:593, 1946.

Finkelstein, H.: Über alimentare Intoxication im Säuglingsalter. Jahrbuch f. Kinderh., 65:1 and 263, 1907.

Ferrant, M.: Methemoglobinemia. J. Pediat., 29:585, 1946.

Govan, G. D. and Darrow, D. C.: The use of potassium chloride in the treatment of the dehydration of diarrhea in infants. J. Pediat., 28:541, 1946.

Hamilton, B., Kajdi, L. and Meeker, B.: The acidosis of acute diarrhea in infants. Am. J. Dis. Child., 38:314, 1929.

Hartmann, A. F.: The effects of diarrhea, vomiting, dehydration and oliguria on the acid-base equilibrium of the plasma of infants with mastoiditis. Am. J. Dis. Child., 35:557, 1928.

Hoag, L. A. and Marples, E.: The acid-base status in dehydration accompanying diarrhea in infants. Am. J. Dis. Child. 42:291, 1931.

Holt, L. E., Courtney, A. M., and Fales, H. L.: The chemical composition of diarrheal as compared to normal stools in infants. Am. J. Dis. Child. 9:213, 1915.

Howland, J. and Marriott, W. M.: Acidosis occurring in diarrhea. Am. J. Dis. Child. 11:309, 1916.

Karelitz, S.: Treatment of toxicosis with the aid of a continuous intravenous drip of Dextrose solution. Am. J. Dis. Child. 42:781, 1931.

Marfan, A. B.: Les orinines de la diete hydrique. Le Nourisson, 25:25, 1937.

Meyer, L. F.: Über den Stoffwechsel der alimentären Dekomposition. Jahrb. f. Kinderh., 71:379, 1910.

Rapaport, S. and Dodd, K.: Intravenous alimentation of infants. J. Pediat., 28:758, 1946.

Schiff, E., Bayer, W. and Fukuyama: Zur Pathogenese der Ernährungsstörungen des Sauglings. Jahrb. f. Kinderh., 119:179, 1928.

Schmidt, K.: Characteristik der epidemischen Cholera, gegenüber verwandten Transsudationsanomalien. G. F. Reyers, Verlagsbuchhandlung. Leipzig-Mitau, 1850.

Schloss, O. M. and Stetson, R. E.: The occurrence of acidosis with severe diarrhea. Am. J. Dis. Child., 12:218, 1917.

Steinitz, F.: Zur Kenntnis der chronischen Ernährungsstörungen der Säuglinge. Jahrb. f. Kinderh., 57:689, 1903.

Tobler, L.: Über Veränderungen im Mineralstoffwechsel des Säuglingskörpers bei akuten und chronischen Gewichtsverlusten. Jahrb. f. Kinderh., 73:566, 1911.

Tobler, L.: Zur Kenntnis des Chemismus akuter Gewichtsstürze. Arch. f. exper. Path. u. Pharmak., 62:431, 1910.

Wilhelmi, A. E., Russel, J. A., Engel, M. G., and Long, C. N. H.: Some aspects of nitrogen metabolism of liver tissue from rats in hemorrhagic shock. Am. J. Physiol., 144:674, 1945.

Winkler, A. W., Danowski, T. S., and Elkinton, J. R.: The Role of colloid and of saline in the treatment of shock. J. Clin. Invest., 25:220, 1946.

PYRIDINE-PROTEIN SILVER METHOD OF DEMONSTRATING NERVE FIBERS*†

GLENN A. DRAGER

INTRODUCTION

The older silver techniques of staining nervous tissue in the whole block have been largely replaced in recent years by methods of impregnating paraffin sections. Of the latter techniques, the Bodian method of activated Protargol (Bodian, 1936 and 1937) is perhaps the most reliable and widely used. However, in the routine use of the Bodian method, most laboratories have encountered uncontrollable variations in the depth of staining tissue elements on the slide other than nerve fibers. Often, the affinity of connective and other tissues for silver results in a very dark background, giving very little contrast between the extraneous tissues and the nerve fibers.

Originally, the Bodian method was designed for the use of the medicinal Protargol prepared by Winthrop Chemical Company. Within the past year or two this company has modified the composition of Protargol, and their product now marketed as Protargol is not suitable for nerve staining. At the present, however, the Winthrop Chemical Company is also manufacturing a strong protein silver purposely designed for tissue staining.

It was found in this laboratory that the protein silver now obtainable necessitated various modifications of the Bodian technique, both in the strength of the solutions and also in the time of exposing the tissue to the solutions. In addition, it was found that the use of pyridine greatly enhanced the silver impregnation. Pyridine has long been used for staining nervous tissue in the block but is not routinely employed in the staining of paraffin sections with silver.

---

*From the Department of Anatomy, The University of Texas Medical Branch, Galveston. This investigation was supported in part by a grant from the Committee on Research in Endocrinology, National Research Council. Received for publication November 4, 1946.

†Thanks are due to Dr. John G. Sinclair for helpful suggestions and criticisms and to Mrs. Nellie Dempewolf for technical assistance.

The following method was particularly designed for demonstrating nerve fibers and terminals in the anterior lobe of the hypophysis; however, the technique is equally good for nerve fibers in other tissues.

Procedure

Only clean glass containers should be used. The extreme care of cleaning glassware with cleaning fluid as emphasized by Cowdry (1943) was not found to be necessary. Water-cleaned glassware gives equally good results. It is important, however, that only glass and not metallic containers be used. All solutions should be prepared with glass-distilled water; however, it is not necessary to wash in glass-distilled water. The tissue to be stained should be removed soon after the animal is sacrificed. After removal of the desired tissue, it is treated in the following manner:

(1) Fix mammalian and warm blooded tissues for 36 to 48 hours in a solution containing:

80% alcohol	90 cc.
Glacial acetic acid	5 cc.
Neutral formalin (40%)	5 cc.
Chloral hydrate	5 gm.

For cold-blooded tissue, the above solution saturated with picric acid was found to be a good fixative, particularly for fish brains. Secondarily, Bouin's fluid gives good results.

(2) If the chloral-formo-acetic-alcohol fixative is used, wash the tissue in many changes of distilled water until no odor of acetic acid remains—usually 4 to 6 hours.

If picric acid is used, wash the tissue in many changes of 50% alcohol.

(3) Place the tissue in 80% alcohol for 10 hours or longer.

(4) Place the tissue in pure pyridine for 10 hours or longer.

(5) Wash the tissue in many changes of distilled water until the odor of pyridine cannot be detected.

(6) Dehydrate in alcohol, clear in cedar oil, embed in 58°–60° paraffin and section at 10 micra.

(7) Remove the paraffin in xylol and run the slides through the alcohols down to water. Place the slides (in glass trays containing 10 slides) for 24 hours at 35° C. in a solution of 3% strong protein silver (Winthrop Chem. Co.) containing about 100 gms. of BB size copper shot (Baker Chem. Co.) per 100 cc. of solution. This is best achieved by placing the very clean copper shot in the staining dish containing glass-distilled water (usually about 200 cc.), then place the glass tray containing 10 slides in the water, permitting the tray to rest on the shot. Lastly, sprinkle the protein silver on the surface of the water, allowing it to go into solution without stirring.

(8) Rinse, *do not wash,* the tray of slides in distilled water. Reduce for 10 minutes in a solution of:

Hydroquinone	2 gm.
Sodium sulphite	10 gm.
Distilled water	100 cc.

(9) Wash thoroughly in many changes of distilled water and replace for another 24 hours in a bath of protein silver and copper as in step 7.

(10) Reduce and wash thoroughly as in step 8. In steps 7, 8, 9 and 10, the slides may be handled in glass trays containing 10 slides; in the following two steps, each slide should be handled individually.

(11) After step 10, the sections are a deep brown color. Place one slide at a time in 1% gold chloride solution containing 2 or 3 drops of glacial acetic acid per 100 cc. Leave the sections in the gold solution just long enough to decolorize the section. In other words, just long enough to remove all of the brown color. This usually requires but a few seconds. The gold solution should be aged a week in a clear glass bottle before using. For certain tissues, gold chloride without the acetic acid is more desirable.

(12) Dip, *do not wash,* in distilled water. Change the water if color appears.

(13) Place each slide in a 1% oxalic acid solution for several seconds or up to a minute. Remove the sections as soon as the faintest bluish tinge appears.

(14) Dip, *do not wash*, in distilled water. Change the water if color appears.
Dipping, and not washing, in steps 12 and 14 are in accordance with Truscott's findings (1944).

(15) Place the slide in a solution of 5% sodium thiosulphate for 10 or more minutes. If the sections turn to bluish purple in this solution, then reduce the time in the oxalic acid. A very faint bluish tinge is desirable. For the sodium thiosulphate solution a staining dish admitting a glass tray holding 10 slides works nicely. Following step 14, the slide may be placed in the sodium thiosulphate solution and another slide started at step 11.

(16) Wash thoroughly in running tap water for at least 10 minutes, dehydrate, clear in xylol and mount in Clarite.

With the exception of the fixative, the first five steps are essentially from the Larsell method of staining nervous tissue (See Rasmussen, 1938, for a detailed description of Larsell's technique). Steps 7 through 16 are essentially the Bodian method (Bodian 1936 and 1937) with the exception of strength of solutions and the length of time the tissue remains in each solution.

Discussion and Conclusions

The original use of pyridine in the staining of nerve fibers is not exactly known. One of the earliest references of its use in microscopic technique is by A. de Souza in 1887. It was then used as a reagent to harden, dehydrate and clear at the same time. He states that it gave particularly good results with brain. Donaggi (1904) used pyridine in a method of demonstrating neuro-fibrils. In 1909, Bielschowsky found that pyridine insured sharper staining of axis cylinders while neuroglia and connective tissue would stain but faintly. This property of pyridine was found also in our laboratory. On

figure 1, it is evident that non-nervous tissue has very little affinity for the silver and gold. In an article describing the various silver methods, Cajal (1910) mentions the use of pyridine and groups it with chloral hydrate, veronal, nicotine, ethylamine, antipyrine as "accelerators." In addition, he states that pyridine, ammonia, veronal and chloral hydrate act as "rejuvenators," increasing the susceptibility of silver impregnation in tissues which had long been fixed in alcohols. Undoubtedly, Cajal had used pyridine for many years previous to that publication. In 1911, Ranson modified and combined portions of the Bielschowsky and Cajal methods and developed the now widely used Ranson pyridine-silver method. Later, Larsell, using pyridine and alcohol as "accelerators," developed a method of impregnating nerve fibers and terminals in decalcified tissue. In our experience, the Larsell method is one of the most reliable of the whole-block staining procedures and, as stated before, the method described in this report is essentially a modified combination of the Larsell and Bodian techniques.

Bielschowsky (1909) thought that pyridine acted as a fat solvent, thus improving impregnation. Other experiments involving fat and lipoid extraction seems to support this view (Landau, 1924). Since pyridine greatly shrinks the tissue, it may be that this substance extracts certain cellular elements that result in a firmer fiber membrane which in turn enhances silver impregnation. On the other hand, the action of pyridine seems to be most effective in the tissue block before the tissue is embedded and sectioned. Holmes (1943) could see no improvement in axon impregnation following the treatment of paraffin sections with pyridine. This, too, has been our experience.

According to Holmes (1943) the pH of the Bodian Protargol-copper solution changes from 8.2 to 6.6 in 24 hours, yet the same concentration of Protargol (1%) without metallic copper decreases only to 8.0. Without copper nerve fiber impregnation is very poor. Obviously, the optimum pH for maximum impregnation is below a pH of 8.0; however, the optimum pH value changes with the fixative used.

Chloral-formo-acetic-alcohol is a relatively acid fixative. The treatment of the tissue with pyridine following such fixation should decrease the acidity and may act to bring the tissue more within the optimum pH range. This is suggested at least from the fact that silver salts are more easily reduced in alkaline solution. Probably the prestaining treatment of the tissue, proper fixation, pyridine, etc., creates physicochemical conditions which nearly approximate optimum conditions for silver impregnation. On the other hand, the metallic copper acts to change the pH of the protein silver bath from alkalinity to acidity. At some point within the range of this pH change, the tissue is subjected to optimum conditions for maximum impregnation.

Summary

A method is described for demonstrating nerve fibers in paraffin sections, using pyridine and protein silver. The discussion includes the historical usage of pyridine and its possible modes of action in enhancing silver impregnation.

LITERATURE CITED

Bielschowsky, M., 1909. Eine Modifikation meines Silber impregnations verfahrens zur Darstellung der neurofibrillen. J. f. Psychol. u. Neurol., XII, s. 135.

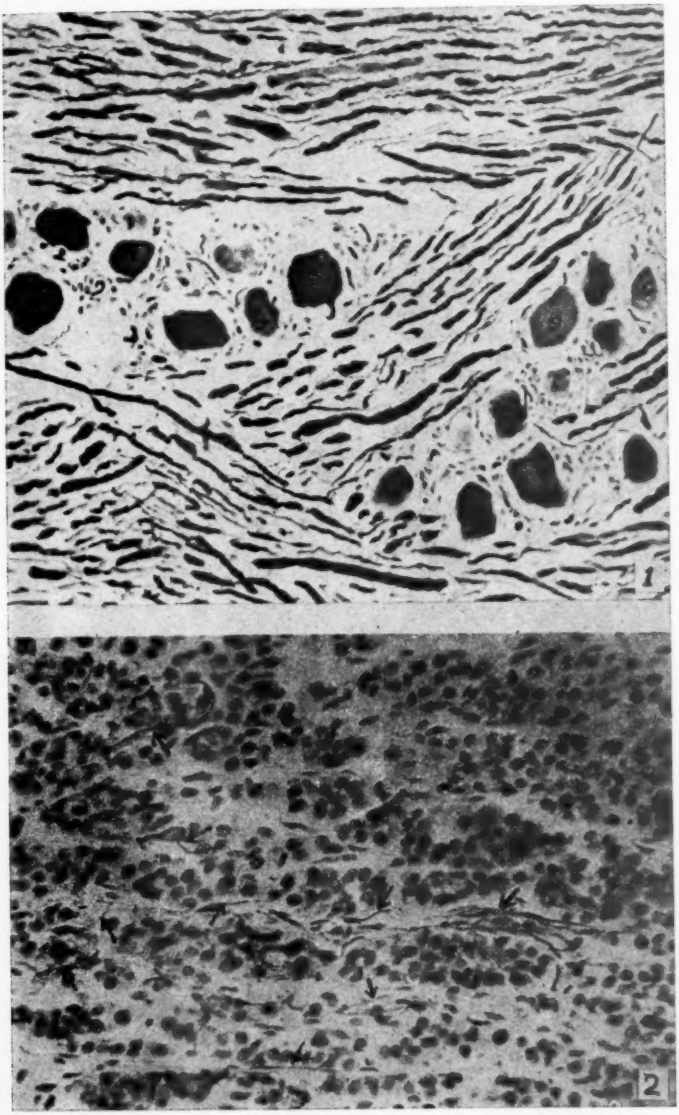
Bodian, D., 1936. A new method for staining nerve fibers and nerve endings in mounted paraffin sections. Anat. Rec., vol. 65, p. 89.

———, 1937. The staining of paraffin sections of nervous tissue with activated protargol. The role of fixatives. Anat. Rec., vol. 69, p. 153.

PLATE 1

Explanation of figures

1. Preparation of the trigeminal ganglion showing cells and nerve fibers. Chloro-formo-acetic-alcohol fixation. X215.
2. Preparation of the anterior lobe of the hypophysis. The arrows indicate nerve fibers. Chloro-formo-acetic-alcohol fixation. X275.


1
2

Cajal, R., 1910. Las formulas del proceder del nitrate de plata reducido y sus efectos sobre los factores integramtes de las neuronas. Trab. Lab. Inv. Biol., Tomo 8, fasc. 1°, y 2°.

Cowdry, E. V., 1943. Microscopic Technique in Biology and Medicine. The Williams and Wilkins Co., p. 35.

Donaggio, 1904 and 1906. Riv. Sper. Freniatr., XXX, p. 397 and XXXII, p. 394. Quoted from Lee's Microtomist's Vade-Mecum, p. 475, 10th ed., P. Blakiston's Son and Co.

Holmes, W., 1943. Silver staining of nerve axons in paraffin sections. Anat. Rec., vol. 86, p. 157.

Larsell, O.—See Rasmussen, A. T., 1938.

Ranson, L. W., 1911. Non-medullated nerve fibers in the spinal nerves. Am. Jour. Anat., vol. 12, p. 67.

Rasmussen, A. T., 1938. Innervation of the hypophysis. Endocrinology, vol. 23, p. 263.

Souza, de A., 1887. Comptes Rendus de Biologie, 8 ser., t. iv., p. 622. Quoted from Lee's Microtomist's Vade-Mecum, p. 56, 10th ed. P. Blakiston's Son and Co.

Truscott, B. L., 1944. The nerve supply to the pituitary of the rat. J. Comp. Neur., v. 80, p. 235.

FURTHER OBSERVATIONS ON THE INTESTINAL BACTERIA OF GUINEA PIGS FED WITH p-AMINOBENZOIC ACID*

DOROTHY M. WHITNEY

The main interest in p-aminobenzoic acid (PABA) has been focused on its antisulfonamide and growth-promoting properties. In addition to these activities detoxicant (Sandground and Hamilton, 1943) and catalytic properties (Henry, 1944) have also been attributed to PABA. Lately, the suppressive action of massive doses of PABA on pathogenic rickettsiae has been established but whether the entire complex of its multiform activities affords an access to the intracellular rickettsiae or whether the rickettsiostatic property is due to one specific function is still obscure.

Since the rickettsiostatic activity of PABA depends largely on its oral administration (Anigstein and Whitney, 1946) it was of interest to investigate the response of the intestinal flora of the treated host to PABA inasmuch as a synthesis of certain substances (folic acid and biotin) by intestinal bacteria has been established (Gant et al., 1943).

In this study special attention was given to gram-negative organisms, the marked reduction of which was previously reported (Whitney and Anigstein, 1946). It may be noted that the disappearance of *Proteus vulgaris* and a proliferation of the acid-forming organisms has been observed in rats fed with PABA (Martin, 1942).

In regard to the fecal flora of guinea pigs, Crecelius and Rettger (1943) report *Lactobacillus* as predominating; *E. coli* was either absent or present in very small numbers. Subtilis-like organisms were found in abundance while gram-negative

*From the Department of Preventive Medicine and Public Health, The University of Texas School of Medicine. Study aided by a grant from John and Mary R. Markle Foundation. The author is grateful to Dr. L. Anigstein, Professor of Preventive Medicine and Public Health for his valuable counsel and constructive advice. The helpful suggestions of Drs. McDonald Fulton and Robert Wise were greatly appreciated. Presented before the Fall meeting of the Texan Branch of the Society American Bacteriologists, Nov. 17, 1946, Dallas, Texas.

non-lactose fermenting organisms appeared in about 50 per cent of the total count. The entercocci were few and the sporulating anaerobes negligible.

Material and Methods

The guinea pigs used in this experiment were obtained from various sources in Texas, and were fed routinely on a diet of chopped cabbage, oats and carrots. When PABA was added, conditions corresponding to the methods and diet used in PABA treatment of experimental spotted fever were duplicated. (Anigstein and Bader, 1946; Anigstein and Whitney, 1946). Two per cent calcium gluconate and ten per cent soya flour were added when indicated.

It is to be noted that, contrary to the previous work (Whitney and Anigstein, 1946) in which PABA was given 2 gm. per day by force feeding, the test animals in the present work were fed ad libitum on the 2 per cent PABA ration (Anigstein and Bader, 1946). One group of test animals received 0.1 per cent PABA to see the effect of low dosage as compared with the higher intake.

Fecal pellets were obtained by collecting specimens dropped on clean paper through wire-bottom cages. Ten per cent suspensions were made in one per cent peptone water and further ten-fold serial dilutions were made in peptone water blanks (Smith and Robinson, 1945). Transfers from these dilutions were made into selected media.

The following media were used: Lactose broth (Difco): One ml. of each serial dilution in peptone water was transferred to 5 ml. lactose broth. Total coliform count was recorded on the basis of the highest dilution in which gas was produced after 48 hours incubation at 37° C.

Nutrient broth (Difco): The same technique was followed in inoculation as used in lactose broth. The total count was estimated as the highest dilution in which growth took place (Smith and Robinson, 1945). Gram stains were made on each culture and the total gram-negative bacilli count estimated as the highest dilution in which gram-negative organisms were found (Poth and Ross, unpublished data).

Desoxycholate agar (Baltimore Biological Laboratories): Poured plates from the serial dilutions were made in order to isolate predominating lactose fermenting organisms.

MacConkey and Endo Agar (Difco): Poured plates from serial dilutions were made to estimate the total number of non-lactose fermenters.

Sodium azide broth for streptococci: Transfers were made from the serial dilutions into 5 ml. sodium azide broth and the total streptococci estimated from the highest dilution in which they were found (Gram stain).

Thioglycollate agar (Baltimore Biological Laboratory): Into 10 ml. of medium, melted and cooled to 45° C., was placed 1 ml. of each serial dilution. The tubes were then kept at 80° C. in a water bath for 15 minutes, cooled and then incubated at 37° C. for 48 hours. The total anaerobic spore count was estimated as the highest dilution in which growth took place.

Mueller-Kauffmann medium: 4.5 gm. of sterilized calcium carbonate add to 90 ml. sterile nutrient broth. After mixing, add 10 ml. 50 per cent sodium thiosulfate, 2 ml. iodine in potassium iodide (20 gm. I in 25 per cent aqueous solution KI), brilliant green (1–1000) 1 ml., ox-bile 5 ml. This is distributed into sterile test tubes. Three tubes of media were inoculated with one-tenth ml. each of the 1–10 fecal suspension. These were then incubated for 48 hours at 37° C. This medium was used especially for the isolation of the Proteus group.

Kligler iron agar, Simmons citrate agar, M.R.-V.P. broth, dextrose, lactose and saccharose broths (All Difco) and 1 per cent tryptone broth were used for further identification. The "imvec" formula of Parr (1939) was used as a basis for coliform grouping.

In addition to the study of the gram-negative bacilli, in which main interest centered, observations on streptococci, and anaerobic spore-bearers were included in order to obtain additional information on the action of PABA.

Results

Intestinal bacteria of untreated guinea pigs

"Total" count: The total number of organisms, estimated from the highest tenfold dilution showing growth in nutrient broth, displayed variations from day to day ranging from 10,000 to 100,000,000,000 per gm. feces. Gram-positive bacteria, of several morphological types, consistently predominated, outgrowing the gram-negative ones in higher dilutions. The "total" gram-negative bacilli, as estimated by Gram stain on the serial broth cultures, usually composed less than one per cent of the total.

Lactose fermenting gram-negative bacilli: The lactose fermenting bacilli as estimated from serial dilution in lactose broth, ranged from 0 to 100,000 organisms per 100 mg. feces representing a span of less than 1 to 10 per cent of the total number of organisms. In the experience of Crecelius and Rettger (1943) the "total" coliform count of the fecal flora of normal guinea pigs varied between 380 and 2,800 per 100 mg. of feces.

In the present study, of the total gram-negative bacilli, the coliform group composes 50 to 100 per cent. As to further analysis, according to the "imvec" formula (Parr, 1939) the predominating gas producer was *Aerobacter aerogenes*, usually accompanied by *E. coli*, and members of the "intermediate" coliform group. Organisms of the proteus type were isolated only in very few instances. In a number of animals it was found that the lactose fermenters seemed to increase following the addition of carrots and cabbage to the diet.

Non-lactose fermenting gram-negative bacilli: The non-lactose fermenters were estimated by counting the colonies which showed no acid production on Endo or MacConkey agar plates. The counts ranging from 1000 to 1,000,000 bacilli per gram feces, included members of the coliform group which showed a slow reaction on carbohydrates. Consequently, these figures are not truly representative of the total non-lactose fermenters; however, these counts may furnish additional

means of comparison of the treated and untreated groups. As to the types of non-lactose fermenters, the predominating organism was a bacillus of the alcalignes type. In addition, a number of unidentified bacilli were found, some of which were glucose fermenters, and some producing yellow, red or green pigments. Contrary to Crecelius and Rettger (1943) who found 50 per cent gram-negative non-lactose fermenters, it was estimated in the present study that the actual non-lactose fermenters composed less than one per cent of the total gram-negative organisms.

Anaerobic spore-bearing bacilli: The serial dilutions in heated thioglycollate agar revealed that the anaerobic spore bearers were usually present in small numbers, ranging from 100 to 10,000 per gram feces.

Streptococci: The streptococci group as estimated by serial dilution in sodium azide broth and checked by Gram stain, were consistently low in number, ranging from 100 to 100,000 per gram feces. These findings roughly correspond to those of Crecelius and Rettger (1943).

Intestinal bacteria of guinea pigs treated with PABA

"Total" count: In the analysis of the bacterial counts in six guinea pigs following 48 hours treatment with PABA, a definite decrease in the "total" count is shown in five of the test animals. For example, reductions from 100,000,000 to 1,000; 10,000,000,000 to 10,000; and 1,000,000,000 to 100,000 per gram feces are recorded. (See Table I GP 10, 12, 16.) Even considering the wide fluctuations in normal animals, the low figures in the PABA treated ones are significant. Both the gram-positive, and the gram-negative organisms are affected, and their relative proportion remains approximately the same as in the untreated series.

Lactose-fermenting gram-negative bacilli: Of this series two animals (10 and 17, Table I) show a striking reduction in this bacterial group, namely from 10,000 to 0, or a 10,000 fold decrease. One animal (15) shows no reaction, two (12 and 14) show a 10 fold, and one (16) a 100 fold decrease,

TABLE I

Bacterial counts per gram feces of guinea pigs before and after 48 hrs. 2% PABA diet

	NO PABA GP 10	2% PABA GP 10	DECREASE	NO PABA GP 12	2% PABA GP 12	DECREASE
TOTAL	100,000,000	1,000	100,000x	1,000,000,000	100,000	10,000x
GRAM NEGATIVE	10,000	100	100x	100,000	100	1,000x
LACTOSE +	10,000	0	10,000x	10,000	1,000	10x
LACTOSE —	10,000	100	100x	100,000	1,000	100x
STREPTOCOCCI	1,000	1,000	0	10,000	1,000	10x
ANAEROBIC SPORES	10,000	100	100x	10,000	1,000	10x
	GP 14	GP 14		GP 15	GP 15	
TOTAL	100,000	1,000,000	0	1,000,000	100,000	10x
GRAM NEGATIVE	100,000	100,000	0	100,000	1,000	100x
LACTOSE +	1,000,000	100,000	10x	100,000	100,000	0
LACTOSE —	100,000	10,000	10x	100,000	10,000	10x
STREPTOCOCCI	10,000	100,000	0	100,000	1,000	100x
ANAEROBIC SPORES	100	100	0	1,000	1,000	0
	GP 16	GP 16		GP 17	GP 17	
TOTAL	10,000,000,000	10,000	1,000,000x	1,000,000	1,000	1,000x
GRAM NEGATIVE	1,000,000	1,000	1,000x	100,000	100	1,000x
LACTOSE +	100,000	1,000	100x	10,000	0	10,000x
LACTOSE —	10,000	1,000	10x	10,000	100	100x
STREPTOCOCCI	10,000	10,000	0	10,000	1,000	10x
ANAEROBIC SPORES	1,000	100	10x	1,000	100	10x

thus three of the series (10, 17, 16) show a significant decrease in the coliform group. Further analysis of this group reveals that the bacterial picture in all six animals of this series had changed; while *E. coli*, which was present in five of the animals prior to PABA treatment, was isolated in four of them after treatment. *A. aerogenes*, present in five of the normal animals, was isolated in only one instance after PABA treatment. None of the other coliform bacilli found in the normal animals were isolated after PABA feeding.

Non-lactose fermenting gram-negative bacilli: These organisms show a consistent reduction in numbers after treatment with PABA (see Table I) but, as previously noted, they also include the slow carbohydrate fermenters of the coliform group. With low figures in the untreated animals the 10 to 100 fold decrease is accordingly not spectacular. The only organism recovered was alcaligines, which appeared in three animals of the series.

Streptococci and anaerobic spore-bearing bacilli: In both of these bacterial groups the counts after 48 hours PABA treatment are within the limits of normal findings.

PABA determinations: As observed in Table II the total PABA intake of guinea pigs treated for 48 hours ranged from 1000 mg. to 2000 mg. per animal. PABA determinations were made on the same fecal suspensions used for bacterial examinations. The total PABA levels obtained (Table II) ranged from 120 to 250 mg. per gm., with the conjugated levels from 65 to 95 mg. per gm., and free PABA from 25 to 165 mg.

TABLE II

PABA values after 48 hours feeding 2% PABA (mg per gm feces)

GUINEA PIG	10	12	14	15	16	17
Total PABA Intake mg.	1800	1200	1600	2000	1600	1000
PABA LEVELS						
free	25	60	160	160	165	115
Conjugated	95	80	80	90	65	75
Total	120	240	240	250	230	190

per gm. feces. As can be seen from Tables I and II, guinea pigs 14 and 15 with little response to PABA in bacterial counts showed PABA levels approximately the same as guinea pigs 10 and 16 showing the most marked response.

Time factor and response to PABA: Twelve guinea pigs were used to test the effect of PABA given for 6, 8 and 10 days; furthermore, five days following the last feeding of the drug after return to normal diet, bacterial counts were repeated. Although in several instances the results seem to indicate a possible change as compared with the untreated series, the figures do not deviate from the normal range. Moreover, the types of bacteria found during the prolonged treatment were similar to those found before PABA feeding. In the group of six guinea pigs fed for 8 days, four of them showed the presence of *Pseudomonas*, an organism otherwise infrequently found. Contrary to the findings of Martin (1942) who noted that the proteus group was inhibited in PABA fed rats, proteus was isolated from four of the animals fed for 8 days. These guinea pigs prior to PABA treatment showed no proteus organisms.

The next problem was to investigate the effect of a prolonged PABA treatment in low dosage to compare with the effect of prolonged treatment with the higher dosage. For this purpose, three guinea pigs were treated with 0.1 per cent PABA and three with 2 per cent PABA for 16 days. PABA was then withdrawn, and the animals returned to normal diet for 5 days. Bacterial counts and PABA determinations were made after 6 and 10 days of PABA treatment and again at the end of the five days of normal diet. The bacterial examination in the 2 per cent PABA fed series revealed no changes as compared with normal animals. The streptococci, in the animals on the low concentration (0.1% PABA) diet showed an increase from 100x to 10,000x. The PABA determinations in the six guinea pigs are tabulated in Table III. Considering that the PABA intake in group II was 20 times less than in group I, the values of free PABA in the former are accordingly lower (Table III); however, the values for the con-

jugated form in the lower dosage group are at least as high as those of the 2 per cent PABA group.

TABLE III

Average PABA values (mg per gm feces) as related to PABA dosage

PABA DOSAGE	length of treatment	PABA values		
		Free	Conjugated	Total
I	6 days	89	30	119
2 gm PABA per 100 gm food daily	10 days	195	24	219
	5 days after PABA removal	49	7	56
II	6 days	8	3	11
0.1 gm PABA per 100 gm food daily	10 days	62	60	112
	5 days after PABA removal	1	17	18

Discussion

The inhibitory effect of massive dosage of PABA on the growth of most of the intestinal bacteria as shown by their decrease after 48 hours of treatment, and the lack of response of certain groups, indicate a selective action. However, this inhibition is of ephemeral character only, as demonstrated by the return to normal levels after 6 to 8 days administration of the drug.

It has been noted that in some animals of the same series, the intestinal bacteria showed a lack of response to PABA, the total counts and their components remaining within normal limits. This phenomenon may be indicative as to individual variations in the response to PABA, the reaction of the bacteria being only one indicator. It remains to be seen to what extent the lack of response to PABA previously observed in certain guinea pigs infected with spotted fever (Anigstein and Bader, 1946; Anigstein and Whitney, 1946) is correlated with these individual variations.

The qualitative analysis of the fecal flora, after the 48 hour feeding period, revealed that *E. coli*, or certain strains of *E. coli*,

show marked resistance to PABA, whereas this organism, shows a marked sensitivity to sulfonamides (Poth and Ross, 1943; Gant et al., 1943). There is an accumulating evidence that *E. coli* is the organism responsible for the synthesis in the intestines of biotin and folic acid or related growth factors. According to Gant et al. (1943), the synthesizing powers of *E. coli* under the influence of sulfonamides may be lost even when their normal numbers are repaired.

As previously noted, PABA when ingested in small quantities, apparently enhances certain groups of bacteria. This would point to a possible stimulating effect of small doses of PABA and would be in line with the concept of the growth-promoting properties of PABA. The relatively high values of PABA in its conjugated form observed in the animals on the low concentration diet are of interest and perhaps of significance. If the conjugation of PABA be considered as a defense mechanism of the animal to transform the drug into its inactive form, the possibility of the stimulating effect of small doses on the tissue to increase conjugation, might be one interpretation of the above phenomenon.

Summary

The investigation of the intestinal flora of guinea pigs before and after feeding with p-aminobenzoic acid is the purpose of this study.

Fecal pellets of guinea pigs before and after treatment with PABA were suspended in 1–10 dilutions of peptone broth and serial 10x dilutions were inoculated into various media. Total bacterial counts were evaluated on the basis of the highest dilutions showing growth in broth. In normal animals, the gram-positive bacteria predominated over the gram-negative. In the former group, bacilli, streptococci, and sporulating anaerobes were found in the order named. Of the gram-negative bacilli, *Aerobacter aerogenes* and *Escherichia coli* dominated the picture. In addition, non-lactose fermenters such as alcaligenes, pseudomonas, and proteus were found.

Guinea pigs treated with 2 per cent PABA in diet for 48 hours showed a marked decrease in number of gram-positive

and gram-negative bacilli. In certain cases, no quantitative reduction was noted, but a definite suppressive and apparently selective action was observed. The 48 hour treatment with PABA resulted in an almost complete inhibition of growth of all gram-negative bacilli with the exception of *E. coli*.

A prolonged feeding of PABA (6–8–10 days) resulted in the return to normal bacterial composition. Small dosage of PABA given over 6 days resulted in increased streptococci counts.

BIBLIOGRAPHY

Anigstein, L., and Bader, M. Anti-rickettsial activity of p-aminobenzoic acid (PABA); The effect of PABA on experimental spotted fever. Texas Reports on Biology and Medicine, 1946, 4; 260.

Anigstein, L., and Whitney, D. M. Anti-rickettsial activity of p-aminobenzoic acid (PABA); Evaluation and significance of PABA blood levels in guinea pigs with reference to spotted fever treatment. Texas Reports on Biology and Medicine, 1946: 4; 338.

Cavill, G. W. K., and Vincent, J. M. Inhibition of mould growth by p-aminobenzoic acid and the n-butyl ester, Nature, 1945, 155; Mar. 10, p. 301.

Crecelius, H. Gilbert, and Rettger, Leo F. The intestinal flora of the guinea pig. Journal of Bacteriology, 1943; 46: p. 1.

Gant, O. K., Ransome, B., McCoy, E., and Elvehem, C. A. Intestinal flora of rats on purified diets containing sulfonamides. Proc. Exp. Biol. and Med., 1943, 52: 276–279.

Henry, Richard J. The mode of action of sulfonamides, Josiah Macy, Jr. Foundation Publication Review Series, 1944, 2: no. 1.

Martin, G. J. The interrelationship of p-aminobenzoic acid and inositol. Am. J. Physiol. 1942, 136: 124–127.

Parr, Leland. The Coliform Bacteria, Bacteriological Reviews, 1939. 3: June, p. 1–48.

Poth, E. J., and Ross, C. A. Bacteriostatic properties of sulfanilimide and some of its derivatives, 1943, 1: 345.

Sandground, J. H., and Hamilton, C. H. Studies on the detoxification of organic arsenical compounds. J. Pharm., 1943, 78: 203–208.

Smith, D. G., and Robinson, H. J. The influence of streptomycin and streptothrycin on the intestinal flora of mice, J. of Bact., 1945, 50: 613.

Whitney, D., and Anigstein, L. The effect of para-aminobenzoic acid on the intestinal flora of guinea pigs. J. of Bact., 1946; 52: 400.

SOME CHARACTERISTICS OF PENICILLIN-RESISTANT STAPHYLOCOCCI

EDWARD H. FRIEDEN, HELEN R. WHITELEY, AND CHESTER N. FRAZIER

The experiments described in this report were undertaken as part of a study of the mechanism of penicillin action. It appeared reasonable to suppose that some light might be shed upon this problem by a comparative study of penicillin-resistant and penicillin-sensitive organisms.

The literature dealing with the development and characteristics of penicillin resistant micro-organisms has already been reviewed (Frazier, 1944; Frieden, 1945). As especially pertinent to the work to be described, brief mention may be made of the following reports:

In 1941, Abraham, et al., found that penicillin-resistant staphylococci, obtained after serial passage on penicillin-containing media, are slower growing than the original culture, and that the rates of liquefaction of gelatin and of coagulation of milk are depressed. It was demonstrated that the resistance to penicillin is not due to the appearance of penicillin-destroying substances. The genetic factors underlying the appearance of penicillin-resistant strains of staphylococci were carefully studied by Demerec (1945). He concluded that the origin of resistors is a true mutation, the appearance of the variant being independent of contact with penicillin. Although the strains studied by Demerec retained their resistance after 20 serial passages in penicillin-free broth, Todd, Turner, and Drew (1945), in a similar study of strains grown in broth containing penicillin, found these to be quite labile, noting nearly complete reversion after relatively few transfers in media free of penicillin.

The present study includes some of the growth characteristics and nutritional requirements of four penicillin-resistant

*From the Department of Dermatology and Syphilology, The University of Texas School of Medicine, Galveston, Texas. Received for publication December 21, 1946.

strains of *Staphylococcus aureus*. These data are compared with the results of similar experiments using the parent (sensitive) strains.

MATERIALS. Nutrient agar: 1.5% bacto-agar, 0.3% beef extract (Difco), 0.5% bacto-peptone, and 0.8% NaCl in distilled water.
Brain-heart agar: 1.5% bacto-agar and 3.7% brain-heart infusion in distilled water.
Brain-heart medium: 3.7% brain-heart infusion in distilled water.
Proteose-peptone medium: 4% proteose peptone No. 3, 0.5% glucose, 1% yeast extract, 1% disodium phosphate in distilled water.
Casein hydrolysate: Pfanstiehl vitamin-free casein was hydrolyzed with boiling 6N HCl for 18 hours, the acid was removed by distillation *in vacuo*, and the residue was dissolved in water, decolorized, and neutralized.
Tryptophane: Pfanstiehl C.P. 1(—) — tryptophane.
1 (—) — Cystine, thiamin hydrochloride, biotin, riboflavin, pyridoxin hydrochloride, and calcium pantothenate were products of Merck and Co.
Nicotinic acid: Eastman Kodak Co.
Inorganic salts: These were either Merck reagent grade, Baker's analyzed, or Mallinkrodt analytical reagent chemicals.
Penicillin: Various commercial lots of penicillin sodium were used. The potency of each product was obtained from the manufacturer and the powder was carefully weighed and dissolved in sterile distilled water to give solutions containing 1,000 units/ml. The solutions were stored frozen in small volumes and used as needed. No variations attributable to differences in origin were noted.

METHODS. Two penicillin-sensitive strains of *S. aureus* were used as parent strains in these experiments. One was the Oxford strain; the other was the F.D.A. standard strain No. 209. Both strains grew profusely on nutrient agar in the absence of penicillin; nearly complete inhibition of growth was observed when penicillin was added at a concentration of 0.02 units/ml.

After several trials, the following procedure was adopted for the development of penicillin-fast variants. One set of nutrient agar plates containing 0.00, 0.02, 0.04, 0.06, and 0.10 units per ml. of penicillin were flooded with an overnight proteose-peptone broth culture of each parent strain. The excess liquid was removed, and the plates incubated for 24–48 hours. From the highest concentrations of penicillin permitting detectable growth, transfers were made to plates of this same penicillin content until, on incubation of the latter, profuse

growth was readily obtained. Transfers were then made to plates containing higher penicillin concentrations, and the process repeated. This procedure yielded, in time, strains which grew moderately well in the presence of 9 units/ml. of penicillin, representing a 900-fold increase in resistance. Attempts to further increase the resistance using these conditions were unsuccessful.

It previously had been observed* that the development of resistant staphylococci was apparently facilitated by the use of brain-heart agar instead of nutrient agar. To confirm this observation, the following experiment was performed: A set of plates containing 0.00, 0.02, 0.03, 0.04, and 0.05 units/ml. of penicillin in nutrient agar was prepared. Another set, similar except that brain-heart agar was used, was also prepared. Sections of each plate were then uniformly inoculated with 0.12 ml. of overnight broth cultures of the two sensitive strains. After incubation for 20 hours, the numbers of surviving colonies were estimated. The results are shown in Table I.

TABLE I

Development of Resistant Strains of S. aureus in Agar

Penicillin Concentration (Units/ml.)	Number of Colonies Surviving			
	Nutrient Agar		Brain Heart Agar	
	Oxford Strain	F.D.A. Strain	Oxford Strain	F.D.A. Strain
0.00	Profuse Growth		Profuse Growth	
0.02	10	2	>100	>25
0.03	0	0	> 25	>25
0.04	0	0	25	12
0.05	0	0	12	2

In view of the results indicated above, brain-heart agar was used as the medium in subsequent experiments. With this modification, no difficulty was experienced in obtaining variants growing well on plates containing 150 units/ml. of penicillin. Although the experiments were terminated at this stage, there appeared to be no reason to suppose that the limit of resistance had been reached.

*By Dr. T. Tung in this laboratory.

To provide an independent check upon these and subsequent observations, another set of penicillin-fast organisms was developed, starting from the same parent cultures. About four weeks were required to obtain variants which grew well on brain-heart agar at penicillin concentrations in excess of 150 units/ml.

Four resistant strains of *Staphylococcus aureus* were finally available for study. Two of these (which will be referred to as A and C) were derived from the Oxford strain of *S. aureus*, while the others (B and D) were variants of *S. aureus* F.D.A. No. 209.

PENICILLINASE PRODUCTION. It is of obvious importance to determine whether or not the resistance of the penicillin-fast organisms used in this study was due to the production of penicillinase. The method of testing used was essentially that described by Gots (1945). Agar plates containing concentrations of penicillin just sufficient to inhibit the growth of a penicillin-sensitive organism are uniformly inoculated with the latter, following which the organism to be tested is streaked (or spotted) upon the plate. After incubation, production of penicillinase is indicated by satellite growth of the sensitive form around colonies of the resistant organism. Using penicillin concentrations of 0.02–0.10 units/ml., with *S. aureus* Oxford as the indicator organism, no evidence could be found for penicillinase production by any of the four organisms tested.

RETENTION OF RESISTANCE UPON SUBCULTURE IN PENICILLIN-FREE MEDIA.

A. In Agar.

In view of the divergent results of previous work on the retention of resistance upon subculture, it was of interest to determine the stability of the strains studied. For studies in agar, two series of experiments were performed, using, respectively, nutrient agar, and brain-heart agar plates. From cultures of strains A-D grown in the presence of 150 units/ml. of penicillin, subcultures were made onto penicillin-free agar.

After incubation for 1–2 days, a second transfer to a fresh plate was made. The first plates were then stored in the refrigerator, and the second series incubated. The first five and the tenth transfer were replated onto brain-heart agar containing 0, 20, 40, 60, 80, and 100 units per ml. of penicillin, in order to determine the sensitivity at these stages. The data for the fifth and tenth transfers are shown in Table II:

TABLE II

Titrations of Resistant Strain Subcultures

No. of Transfers	Strain	Nutrient Agar Penicillin, Units/ml.						Brain-Heart Agar Penicillin, Units/ml.					
		0	20	40	60	80	100	0	20	40	60	80	100
5	A	4	4	4	4	4	4	4	4	4	4	4	4
5	B	4	2	2	3	1	2	4	4	2	2	1	1
5	C	2	3	3	3	2	2	4	3	3	4	3	3
5	D	4	1—	1—	1	1—	1—	3	3	3	3	3	3
10	A	4	3	3	3	3	3	4	2	2	1—	—	—
10	B	4	4	4	3	3	3	4	1	2	—	—	—
10	C	4	4	4	1	3	3	4	4	3	3	2	2
10	D	4	—	—	—	—	—	4	2	1	1	—	—

(Extent of growth is indicated by the numbers, decreasing in the sequence 4, 3, 2, 1, —.)

Table II indicates that five passages on brain-heart agar has but little effect on the resistance of all the strains studied. Except for strain D, this is also true for subculture on nutrient agar. After ten passages on brain-heart agar resistance is substantially decreased for all but strain C, whereas, again with strain D as an exception, passage on nutrient agar does not appear to have as much effect on the resistance to penicillin. In general, it would appear that with strains, A, B, and C resistance is maintained more readily on nutrient agar as the culture medium. This may perhaps be explained by assuming that the richer brain-heart substrate facilitates the appearance of "reverts" which may then crowd out the others because of

their faster growth. A different explanation must, however, hold for the data with respect to strain D.

B. In Liquid Media.

Brain-heart broth was used, since preliminary experiments had indicated that the resistant strains grew more rapidly in this than in proteose-peptone medium. Five ml. volumes of broth were inoculated with loop scrapings from plates on which the resistant strains had been grown. After incubation in the water bath for 24 hours, transfers were made to fresh media. This was repeated for a total of 6 transfers. The sensitivity of the sixth passage was compared with that of a fresh first transfer. As before, the sensitivity was determined by titration on brain-heart agar. The data are summarized in Table III:

TABLE III

Retention of Resistance After Subculture in Brain-Heart Broth

No. of Transfers	Strain	Penicillin Concentration, Units/ml.				
		0	20	40	60	80
1	A	4	3	3	4	4
1	B	4	4	4	4	4
1	C	3	4	4	3	2
1	D	4	3	4	4	3
6	A	4	3	3	3	2
6	B	4	1	—	—	—
6	C	4	1	—	—	—
6	D	4	—	—	—	—

(The notation is the same as that of Table II)

With the exception of strain A, resistance is rapidly lost upon broth subculture.

Growth Characteristics. Perhaps the most striking characteristic of the four penicillin-fast staphylococci studied was the marked depression in the rate of growth as compared to the parent strains. Thus, in proteose-peptone or brain-heart

broth, good growth was obtained only after 24–48 hours' incubation. The levels of growth, as determined by photoelectric evaluation of turbidities, never reached those attained by the parent strains in 12 hours or less under these conditions.

In addition to the relative paucity and slowness of growth, the resistant variants were also characterized by the fact that the results of experiments on growth rates or inhibition curves were subject to a considerably larger degree of variation than were similar experiments using sensitive strains. This difficulty could be overcome in part by using scrapings from penicillin-free slants as inocula; from the data already presented, it is apparent that no loss in resistance occurs after a single transfer to penicillin-free agar.

Growth rates and inhibition curves were studied turbidimetrically in liquid media. Three media were used: proteose-peptone broth, brain-heart broth, and a complete synthetic medium. The latter contained hydrolyzed casein (1.0%), glucose (0.5%), cystine (0.01%), tryptophane (0.01%), thiamine hydrochloride (1.0γ/ml.), nicotinic acid (1.0γ/ml.), $FeSO_4.7H_2O$ (0.001%), $MgSO_4.7H_2O$ (0.02%), K_2HPO_4 (0.2%), and sodium acetate (0.2%) in distilled water. In 24 hours, both original strains showed fairly abundant growth on the synthetic medium. This mixture will also support growth of the resistant strains, although the levels reached are below those attained by the parent strains. Growth of the resistant forms is enhanced by the addition of a mixture of biotin (0.02γ/ml.), riboflavin (1γ/ml.), pyridoxin (1γ/ml.), and calcium pantothenate (1γ/ml.); none of these appear to be essential, however, nor does their addition stimulate the growth of the resistors to the levels attained by penicillin-sensitive forms under like conditions.

Growth curves for strains A, B, C and D are shown in figures 1–3. Except for the very early parts of the curves, each point represents the average of duplicate or triplicate readings. Attention may be called to three points worthy of note:

1. The rate of growth in all cases is seen to be relatively quite slow. Under similar conditions, the parent strains afford nearly maximal growth in about 12 hours in proteose-peptone and brain-heart broth.

2. The most rapid, as well as the most abundant growth occurs in proteose-peptone medium. Both the other media are definitely less favorable.

3. Considerable strain to strain variation is encountered in complex media. In the simpler synthetic medium the differences, especially in rate of growth, are not as pronounced.

INHIBITION BY PENICILLIN IN LIQUID MEDIA. Penicillin inhibition curves are shown in figures 4–6. These curves represent duplicate turbidimetric measurements of the growth levels attained in 36 hours in media containing 0.00, 0.01, 0.10, 1.0, 10.0, and 50.0 units per ml. of penicillin. (The curve for strain C in brain-heart broth is omitted due to the fact that the mucoid-like growth obtained in this series could not be uniformly re-suspended.)

The data obtained are consistent with the earlier findings on growth rates. Again, the least amount of strain variation is found in experiments employing synthetic media. All of the strains, with C a possible exception, appear to be more sensitive to penicillin in proteose-peptone broth. Since this substrate also promoted more rapid and abundant growth, it seems reasonable to assume that this medium facilitates, in some manner, the appearance of penicillin-sensitive revert forms which, in the absence of penicillin, may be expected to grow more rapidly and more profusely.

In Figure 4, there are a few points which seem to indicate that very low concentrations of penicillin can act as a growth-stimulant, rather than as an inhibitor. While these points must naturally be viewed with some suspicion, especially in light of the admittedly increased experimental error involved, it is interesting to note that Miller, Green, and Kitchen (1945) found some evidence for stimulation of *S. aureus* by low concentrations of penicillin.

From a consideration of the data presented, it would appear that no marked difference exists between the nutritional requirements of the sensitive and resistant strains studied. It seemed desirable, however, to check the effect that various components of the synthetic medium might have upon the reversion of resistant to sensitive, less fastidious forms. The following experiment was therefore performed:

A total of seven media were used. Medium No. 1 was the complete synthetic mixture described earlier. Media 2–6 were, respectively, deficient in cystine, tryptophane, thiamine, nicotinic acid, and sodium acetate. Medium No. 7 was proteose-peptone broth. Tubes of each medium were inoculated with washed saline suspensions of strains A, B, C, and D (from first-stage penicillin-free slants). After 48 hours' incubation, the cultures were transferred to fresh tubes of the same medium. The resistance of the organisms after the first transfer was determined by plating out onto brain-heart agar containing 0, 20, 40, 60, 80, and 100 units of penicillin per ml. Finally, the levels of growth attained in the liquid cultures were estimated turbidimetrically. This procedure was repeated for a total of four transfers.

As a control experiment, the same procedure was repeated with the parent strains, except that sensitivity titrations were made on plates containing 0.00, 0.01, 0.02, 0.03, and 0.05 units/ml. of penicillin.

The data are summarized in Table IV. It should be noted that the experiments were subject to some uncertainty; sensitivity tests were sometimes ambiguous. It is nevertheless possible to draw the following conclusions:

The resistance of all strains was least affected by passage through proteose-peptone medium. Only strain D showed any appreciable dimunition in resistance after four transfers. Some lowering of resistance, again especially pronounced in strain D, was apparent in medium 1. Medium 2, deficient in cystine, adversely affected the resistance of all but strain C. Strains B and D showed marked reduction in resistance after passage in media 3 and 4, the other strains showing only a moderate

TABLE IV

Effect of Composition of Medium Upon Growth and Resistance of Normal and Penicillin-fast Strains of *Staphylococcus aureus*

Medium	Strain A		Strain B		Strain C		Strain D		Strain O		Strain F	
	Gr.*	Res.*	Gr.*	Res.*	Gr.*	Res.*	Gr.*	Res.*	Gr.*	Res.*	Gr.*	Res.*
Complete synthetic	+	0	0	–	+	0	+	—	0	–	+	–
Cystine deficient	+	–	+	—	0	–	+	—	++	–	++	–
Trytophane deficient	0	–	0	—	0	0	+	—	0	–	+	–
Tryptophane deficient	0	0	0	—	0	0	+	—	0	–	0	–
Nicotinic Acid deficient	Poor growth of all strains											
Acetate deficient	–	–	0	—	0	0	0	–	+	–	+	–
Proteose-peptone	+	0	++	–	++	0	++	–	+	–	0	+

(Data refer to comparison of properties of growth and resistance of strains before and after four serial passages in the liquid media indicated.)

++ marked increase
\+ slight increase
0 no significant change
– slight decrease
— marked decrease
* Growth-Resistance

decrease. Like the parent strains, the resistant ones all required nicotinic acid. In the absence of acetate, the resistance of strain C was apparently unaffected, whereas the others were rendered more sensitive.

An interesting result of the experiment was that continued transfer of strains O and F in nearly all media resulted in a measureable increase in sensitivity to penicillin. These strains ordinarily show moderate growth on plates containing 0.01 units/ml. of penicillin, but after four liquid media passages, the same concentration inhibited nearly all growth.

From the fact that passage in synthetic and proteose-peptone media had approximately similar effects upon retention of resistance, it would appear that the complete synthetic medium satisfies the nutritional needs of resistant staphylococci. Strain B seems to be quite sensitive to the withdrawal of cystine, tryptophane, thiamine, or acetate. While strain D loses resistance rapidly when either cystine, tryptophane, or thiamine is withdrawn, the loss in resistance may not be attributable to these deficiencies, since a comparable loss occurs upon subculture in medium 1. It is, of course, possible that some alteration in the requirements for amino acids other than cystine and tryptophane has occurred; this change would not be detected in these experiments.

Summary

A method has been described for the development of strains of *Staphylococcus aureus* resistant to 150 units of penicillin per ml., representing a ten thousand-fold increase in resistance. A study of four such strains has revealed that the resistance is not due to the production of penicillin-destroying substances, that the resistance is fairly stable to transfer on penicillin-free agar (less stable to transfer in penicillin-free broth), and that the resistant varieties are characterized by a greatly depressed growth rate. Studies of the nutritional requirements of the four strains have failed to demonstrate any obvious differences as compared to those of the parent (sensitive) strains.

REFERENCES

Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., and Florey, H. W.: Further observations on penicillin, Lancet, *2:* 177–189 (1941).

Demerec, M.: Production of *Staphylococcus* strains resistant to various concentrations of penicillin, Proc. Nat. Acad. Sci., *31:* 16–24 (1945).

Frazier, C. N.: Microbial adaptation to penicillin, Texas Rep. Biol. Med., *2:* 385–396 (1944).

Frieden, E. H.: The nature and action of the antibiotics, Texas Rep. Biol. Med., *3:* 569–646 (1945).

Gots, J. S.: Production of extracellular penicillin-inactivating substance associated with penicillin resistance in *Staphylococcus aureus*, Proc. Soc. Exp. Biol. Med., *60:* 165–168 (1945).

Miller, W. S., Green, C. A., and Kitchen, H.: Biphasic action of penicillin and other sulfonamide similarity, Nature, *155:* 210–211 (1945).

Todd, E. W., Turner, G. S., and Drew, L. G. W.: Temporary character of 'fastness' of staphylococci to penicillin, Brit. Med. J., *1:* 111–113 (1945).

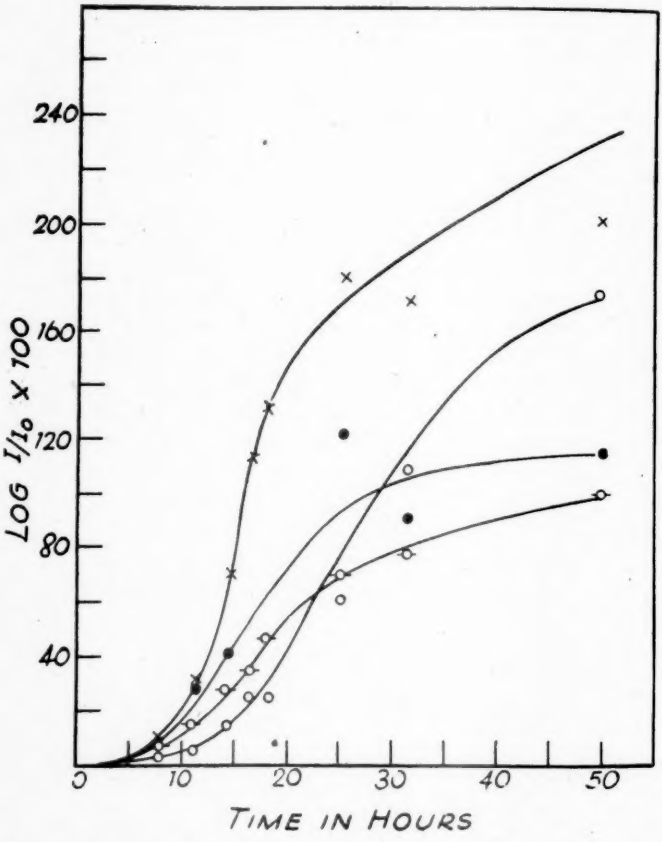


Figure 1: Growth rates of penicillin-resistant staphylococci in proteose-peptone medium. X—strain A; O—strain B; ●—strain C; -O- -strain D.

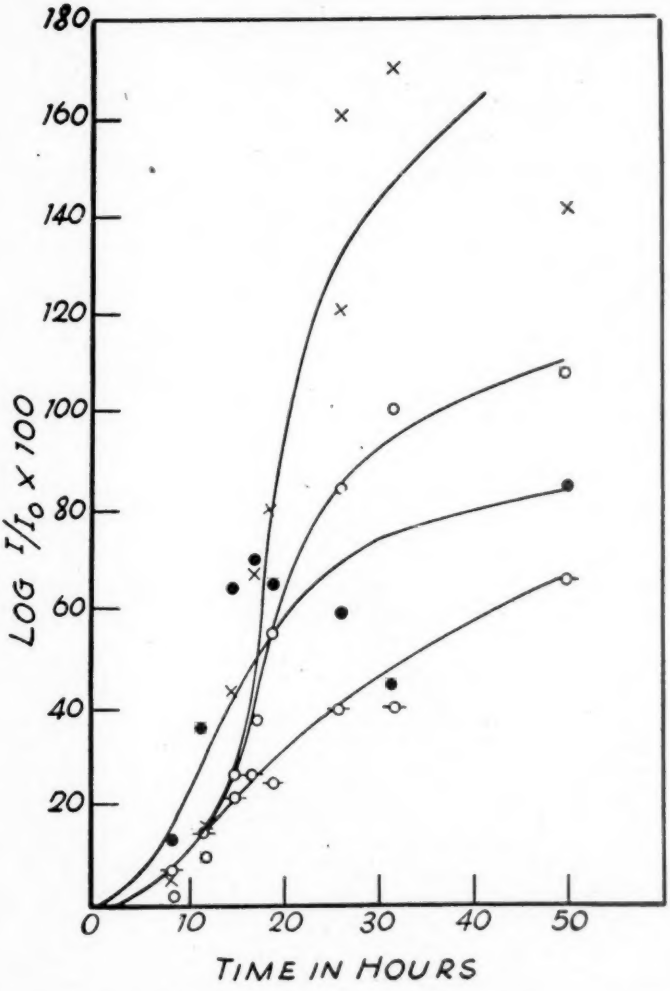


Figure 2: Growth rates of penicillin-resistant staphylococci in brain-heart medium. X—strain A; O—strain B; ●—strain C; -O- —strain D.

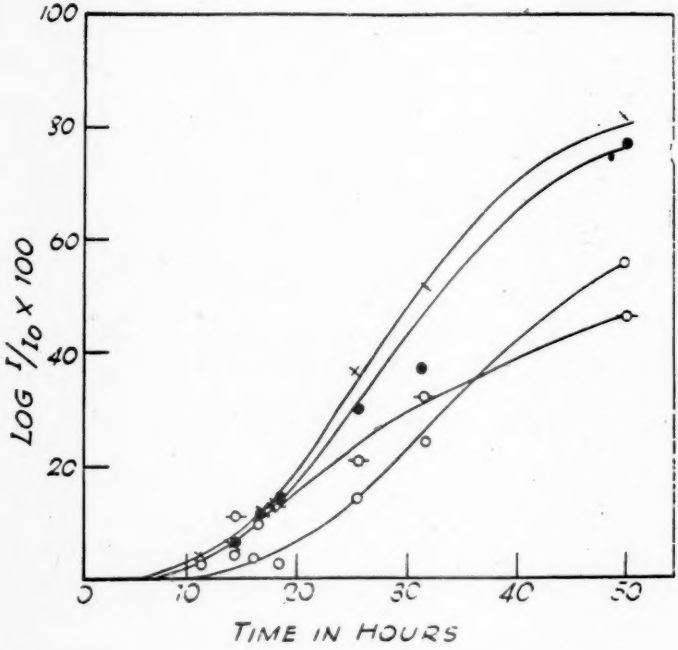


Figure 3: Growth rates of penicillin-resistant staphylococci in synthetic medium. X—strain A; O—strain B; ●—strain C; -O- —strain D.

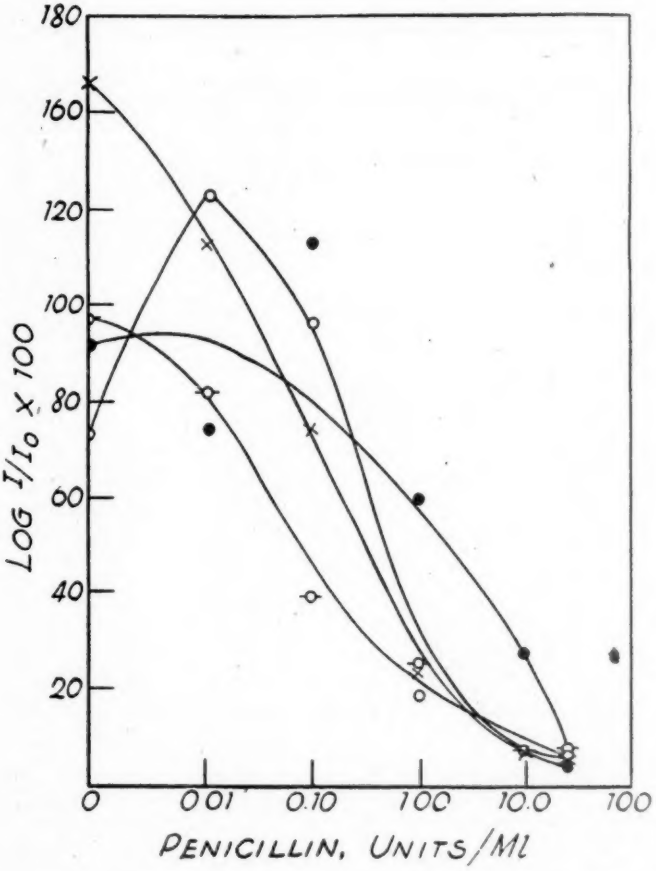


Figure 4: Growth of penicillin-resistant staphylococci in proteose-peptone medium containing penicillin. X—strain A; O—strain B; ●—strain C; -O- -strain D.

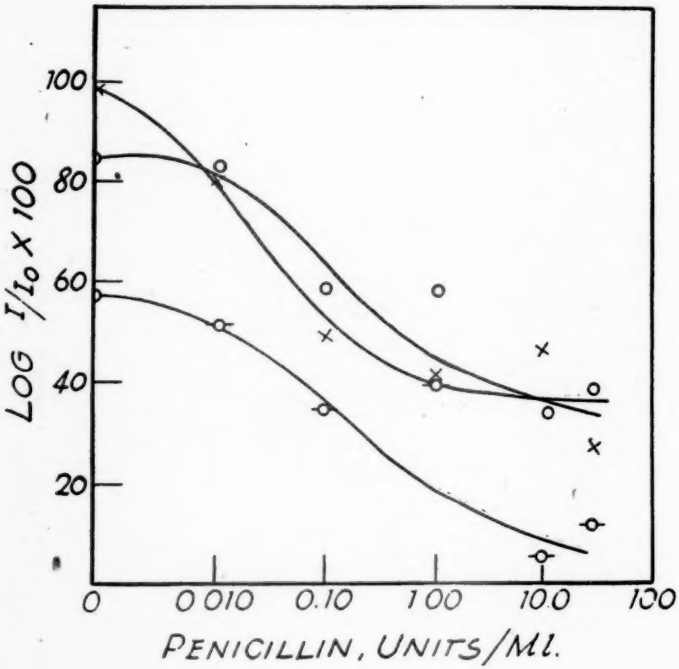


Figure 5: Growth of penicillin-resistant staphylococci in brain-heart broth containing penicillin. X—strain A; O—strain B; –O– —strain D.

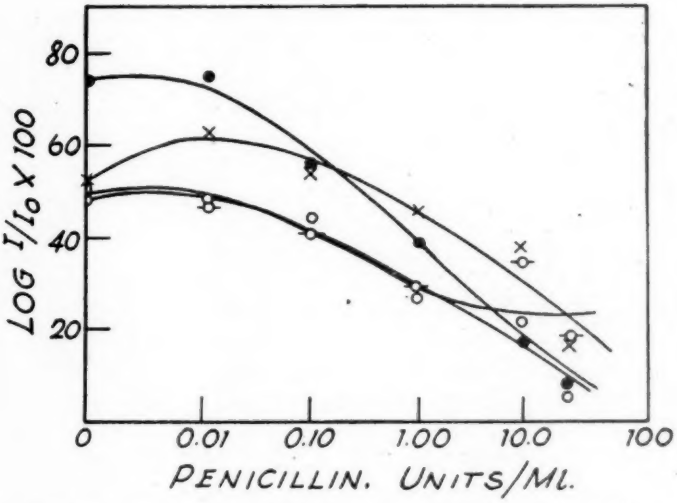


Figure 6: Growth of penicillin-resistant staphylococci in synthetic medium containing penicillin. X—strain A; O—strain B; ●—strain C; -O- -—strain D.

OBSERVATIONS ON CELLS FROM ASCITIC FLUID CULTURED *IN VITRO**

JAMES B. IVERS AND C. M. POMERAT

There are, as is commonly known, many pathological conditions which directly or indirectly give rise to the accumulation of varying amounts of fluid within the peritoneal cavity. The most common causes of this condition can be listed as follows: portal cirrhosis, congestive heart failure, renal failure with edema, neoplastic peritonitis, acute inflammatory peritonitis, tuberculous peritonitis, and chronic constrictive pericarditis. It is obvious that the mechanism of production of this fluid varies greatly with these conditions, and consequently the cellular content of the fluid varies with different causes. For instance, ascites occurring with portal cirrhosis is the result of a portal hypertension, with consequent transudation of fluids into the peritoneal cavity. This is purely a mechanical process involving fluid pressures, therefore the cellular content of the fluid will be low. However, in neoplastic or inflammatory peritonitis there is active migration of cells into the peritoneal cavity and the total number of cells will be increased. Our interest in these studies lies primarily in the observation of cells from ascitic fluid taken from patients having some form of neoplastic peritonitis, these cells having been grown in tissue culture.

Familiarity with the cellular content of the peritoneal fluid can be readily obtained from the study of experimental exudates (Wagoner and Custer, 1932). Using such material Maximow (1927) provided an excellent description of the appearance and behavior of the various common cell types. Weitzmann (1938) appears to have given the first report of tissue cultures of cells from human peritoneal and pleural fluids. He observed rounded clusters of cells from a case of

*Aided by a grant from J. B. Roerig and Company. Contribution from the Tissue Culture Laboratory, Department of Anatomy, and Galveston State Psychopathic Hospital, The University of Texas Medical Branch. Received for publication January 2, 1947.

pleural carcinomatosis and characteristic malignant immature lymphocytic elements from the ascitic fluid of a lymphosarcoma. Unfortunately the article has no bibliography.

Recently Coman (1943) briefly described the growth of cells from non-malignant and malignant peritoneal and pleural effusions. He gave evidence that free floating neoplastic cells remain viable and can multiply following attachment to the substrate provided by the culture. This shows that vascular and lymphatic dissemination is not obligatory. He suggested that colonies of malignant elements might be produced from a single cell.

Ascitic fluid was collected under strict aseptic conditions.* Maintaining sterile technique at all times this fluid was placed in 250 cc. centrifuge bottles and centrifuged at 1500 r.p.m. for 15 minutes. The cells were then collected from the bottom of the bottle with long pipettes and placed in a small tube. For roller tube culture (c.f. Gey, 1933) they were usually resuspended in their own supernatant fluid. For hanging drop cultures the cells were suspended in rooster plasma. The roller tubes used had a constriction about 3 cm. from the bottom. Into the part of the tube limited above by the constriction, 0.1 cc. of heparinized rooster plasma and 0.1 cc. of cell suspension were introduced. This mixture was spread over the walls of the tube. Then 0.1 cc. of extract made from chick embryos incubated for 7 days was added and the tubes were rapidly rolled to provide even distribution. The embryonic extract clots the plasma and fixes the cells in a solid medium. The tubes were allowed to stand 1 hour and then a supernatant fluid was added and they were placed in a holder which rotated each tube ten times per hour. Nutrition is thus provided on a simple diffusion basis and effective elimination of waste products was provided by changing the supernatant fluid every 3–4 days. The supernatant used in these experiments consisted of 10 parts ascitic fluid, 10 parts Tyrode and one part chick embryonic juice. Ascitic fluid was available in large

*We are indebted to Dr. A. O. Singleton and Mr. John K. Cox for their interest and kind coöperation in this project.

quantities and it appeared effective even after storage for three months in the ice box. We believe ascitic fluid as a component for nutrient media for tissue cultures is worthy of further study. Roller tube cultures, in our hands, have been maintained more than 3 months using this method.

Hanging drop cultures were prepared by placing 1 small drop of plasma containing the cells upon a coverslip. To this 1 drop of embryonic extract was added and the preparation was mixed thoroughly. This was then set up by usual hanging drop method, paraffin sealed and placed in an incubator at 37° C. The cultures survived until the cells exhausted the nutritional supply. Healthy looking cells have been seen for as long as 10 days. In addition to day-to-day examination in the living state, usually three cultures were picked at random for fixation. The fixative used was Helly-Zenker solution. The stain used was Delafield's haematoxylin with a counterstain of Eosin-Azure II.

It is immediately evident that if we are to study abnormal cells occurring within the peritoneal cavity, we must be able to recognize the normal cells which can be seen in any form of ascites. These cells fall into two groups, blood and tissue elements. Whenever any ascites occurs, there will be a varying number of leucocytes migrating into the abdominal cavity. In many instances this is undoubtedly a passive migration; in others it is active. The leucocytes occurring most frequently will be polymorphonuclear leucocytes, lymphocytes, and monocytes. The polymorphonuclear leucocytes are easily recognized. The lymphocytes in the early stages are also readily identified. However, after 12 to 24 hours of incubation in the tissue culture, these cells are in the process of transforming into a cell resembling a monocyte and then will go on to form a typical exudative polyblast or macrophage. The final stage of transformation is a fibroblast. The monocyte will follow the same course. This transformation has been clearly worked out and is described in Downey's Handbook of Hematology (1938) in a chapter written by Bloom.

Of the cells classified as tissue elements two types will commonly be seen, tissue macrophages or exudate polyblasts and

mesothelial cells. Macrophages will assume the polyblastic appearance earlier than cells derived from lymphocytes or monocytes. Also noted are the mesothelial cells of the peritoneal lining, not to be confused with endothelial cells. According to Maximow (1927) free rounded mesothelial cells do not show phagocytosis, accumulate a very small amount of vital dye and never transform into polyblasts. Mesothelial cells have a large, folded pale nucleus with large nucleoli and fat-like droplets in a basophilic cytoplasm. They are very sensitive to mechanical injury. In cultures mesothelial cells ultimately transform into typical fibroblasts but their origin can be traced through a stage in which they have long, string-shaped, branched cell bodies and knot-shaped nuclear swellings. Exudative polyblasts are of the large round mononuclear type with rather prominent dense, wrinkled nuclei and basophilic cytoplasm. There may be one to three nucleoli. These cells in tissue culture quickly transform into a spindle-like cell that closely resemble young fibroblasts. As these cells differentiate in the medium, they tend to assume an embryonic appearance and it is not uncommon to see multinucleated cells of this type. The difference between peritoneal polyblasts and desquamated mesothelial cells can be seen in a figure on page 67 of Maximow-Bloom's Textbook of Histology (1943).

It is obvious that when studying cells taken from patients with various types of neoplastic peritonitis one will see a great multitude of different types which, as yet, cannot be readily classified. However, as we shall show, it is often easy to demonstrate the presence of cells and cell formations that definitely do not resemble in any way the elements seen in fluid of non-neoplastic patients. But, as might be expected, frequently cells believed to be of neoplastic origin will very closely resemble normal ones. This is more evident when we recall that a great many of the criteria of malignant cells are based upon their embryonic appearance. Therefore, the differentiation of a sarcoma cell from a rapidly growing normal mesothelial cell is an uncertain procedure. It is even more difficult in view of the fact that in many instances these

cells are set up in tissue culture as a suspension of free elements and when examined it is necessary to study the individual cell. In the absence of adjacent normal tissue the difficulties of neoplastic diagnosis are very much increased; indeed, diagnoses on individual cells from ascitic fluid are considered unreliable by many pathologists. Criteria useful for such studies, however, have been summarized by Lewis (1939).

As an example of the type of study where it is difficult to differentiate normal from abnormal cells, is seen in the case of Patient A who had a diagnosis of scirrhous carcinoma of the stomach. He accumulated about one liter of ascitic fluid per week having a specific gravity of 1.010. It was of a clear yellow color and upon centrifugation quite a large amount of cellular material was obtained. These cells were set up in roller tubes and hanging drop cultures. At the initial stage, there was an even suspension of cells (Fig. 1) comprised of polymorphonuclear leucocytes, some lymphocytes, and a great many large round mononuclear cells (Fig. 2). The mononuclear cells had large, usually round, but occasionally folded nuclei. The nucleoli were very dense and prominent and often as many as 2 or 3 in one nucleus. The chromatin network within the nucleoplasm was densely stained. These cells frequently were binucleate, in which case the nuclei practically filled the entire cell. They were presumed to be exudative polyblasts and not giant cells. Multinucleated cells containing from three to six nuclei were also occasionally noted. Within twenty-four hours differential death of the polymorphonuclear leucocytes could be detected, but of more significance was the appearance of large fusiform cells (Fig. 3). Equally important was the appearance of definite changes in the polyblasts noted at the initial phase. Cytoplasmic thread-like processes were seen and many of the cells were becoming pear-shaped. This was interpreted as the beginning transformation stage of the polyblasts into fibroblast-like cells. By seventy-two hours these spindle cells were present in great numbers and dominated the appearance of the cultures (Fig. 4). They were of many shapes and forms, some presenting a very bizarre appearance. They possessed large nuclei, often with 3 to 4

densely staining irregularly shaped nucleoli. The nuclear membrane was very distinct and often the nuclei showed an indentation on one side. The cytoplasm was moderately dense and frequently showed large vacuoles, suggestive of pinocytosis, although this was not observed in the living state. The majority of these fibroblast-like cells had long thin processes, usually two in number and bipolar. Frequently these processes formed interlacing networks with other such cells. Less commonly binuclear tadpole-like forms were noted and also cells of an angular shape were seen. A great many showed multinucleation, containing as many as four nuclei. Occasionally large giant cells (Fig. 5) containing 10 to 20 densely staining nuclei were seen. The peak of growth and cytological differentiation was reached by 96 hours in the hanging drop cultures. By this time very few of the transitional cells were seen and it was significant that with the fall in their number the frequency of the fibroblast-like cell increased (Fig. 6). It was also generally evident that after about 100 hours of growth, the spindle cells began to enlarge, the processes becoming less filamentous and much longer. By approximately 150 hours most of the non-spindle elements were dead. This was taken as confirmation of Coman's (1943) statement that the macrophages tend usually to disappear within a week. Since it is well established that macrophages transform to fibroblasts, we must conclude that at least a part of the spindle cells were derived from these elements. By 200 hours the spindle cells were definitely larger (Fig. 7) than earlier and often attained gigantic sizes, some having nuclei measuring 22 microns along the long axis and a total length of one millimeter (Fig. 8). At 216 hours a foamy appearance of the cytoplasm was commonly noted, probably due to fatty droplets. The nuclei had serrated borders and some pyknosis was seen. The study was terminated at this time.

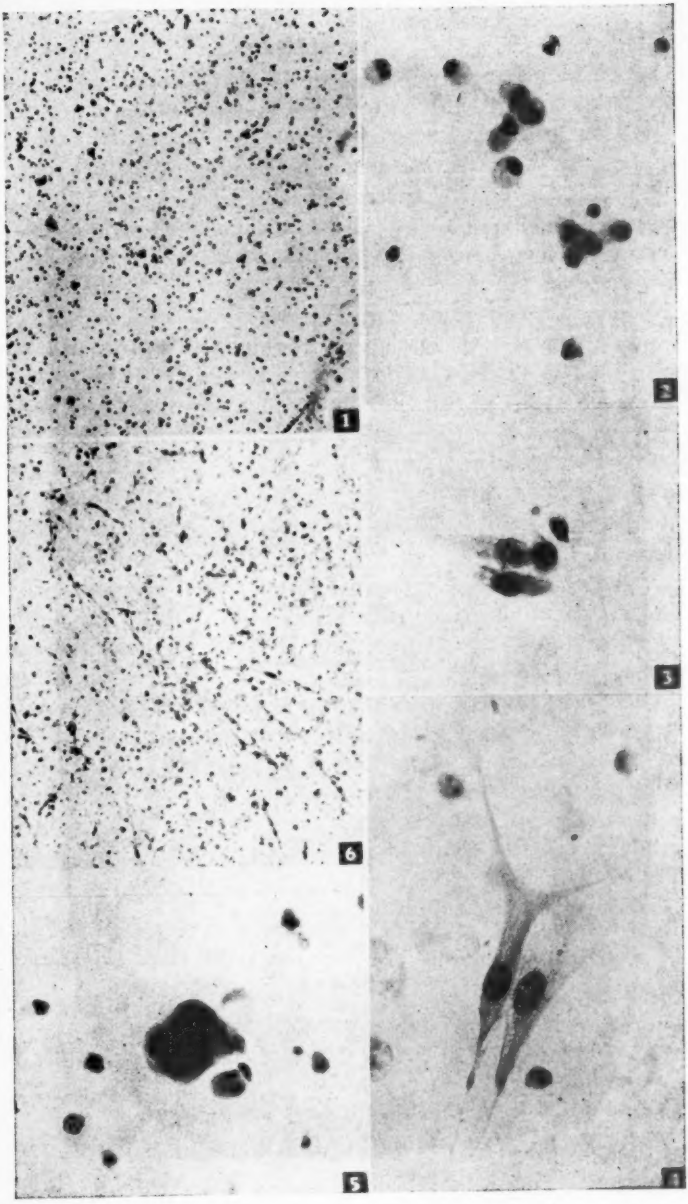
Even though such a description is likely to give the impression that we believe these cells to be malignant, we cannot draw that conclusion. While many of the characteristics of the cells of Patient A would be compatible with a diagnosis of malignancy many of these same features appear in proven

non-malignant cells. For instance, the transformation from round cells to spindle cells, multinucleation, vacuolization, large multiple nucleoli, and hyperchromaticity appear in normal transformed polyblasts. Unfortunately, laparotomy was not performed nor was a postmortem examination obtained on Patient A. Therefore, it is possible that ascites may have been present upon the basis of metastatic lesions in the liver with partial portal obstruction, and also that malignant cells may not have been present in the peritoneal fluid. Further study of ascitic fluid of gastric carcinoma with tissue culture technique is highly desirable. It is noteworthy that even when the growth was most pronounced mitotic figures were not seen which would indicate that the cells were not actually proliferating.

A useful check of the conclusion that Patient A did not demonstrate a cellular picture pathognomonic for gastric carcinoma was provided by Patient B, diagnosed as cirrhosis of the liver with ascites. In this subject the fluid was clear and straw colored with a specific gravity of 1.012. Upon centrifugation there were cells present, but not nearly as many as obtained from Patient A. These were also set up in roller tubers and hanging drop cultures. Initially a suspension (Fig. 9) comprised of polymorphonuclear leucocytes in small numbers, many lymphocytes and many large round mononuclear cells was seen. No giant cells or other multinucleated types were noted. The large mononuclear elements had the appearance of mesothelial cells (Fig. 10). Many polyblast-like cells with thready processes were seen which displayed active ameboid movements. By 15 hours of growth a few fusiform cells were seen and many classical polyblasts (Figs.

Figures 1–6. Hanging drop cultures of cells from ascitic fluid from Patient A. Hematoxylin-Eosin-Azure.

Figure 1. Initial stage. 100X. Fig. 2. Same as Fig. 1 at 500X. Fig. 3. Beginning spindle formation at 24 hours. 500X. Fig. 4. Spindle cells at 72 hours. 500X. Fig. 5. Multinucleated giant cell at 120 hours. 500X. Fig. 6. Spindle cells and disintergrating elements at 120 hours. 100X.


1
2
3
4
5
6

11 and 12). Rarely the fusiform cells were binucleate. The transitional forms noted in Patient A were also seen. From this time on the cultures of Patient B very closely followed the general characteristics noted in Patient A. The polyblasts tended to disappear between 100 to 150 hours and with increasing age the spindle cells became much larger, the majority of them having the appearance of typical fibroblasts (Fig. 13). As was noted in Patient A the only cells left by 150 hours were the large spindle types. Thus it is evident that two cases representing ascites of neoplastic and non-neoplastic origin may be extremely similar in tissue culture. The only real differences in our study was the presence of occasional multinucleated giant cells seen in Patient A and not in Patient B; and the greater frequency of multinucleated spindle cells in Patient A.

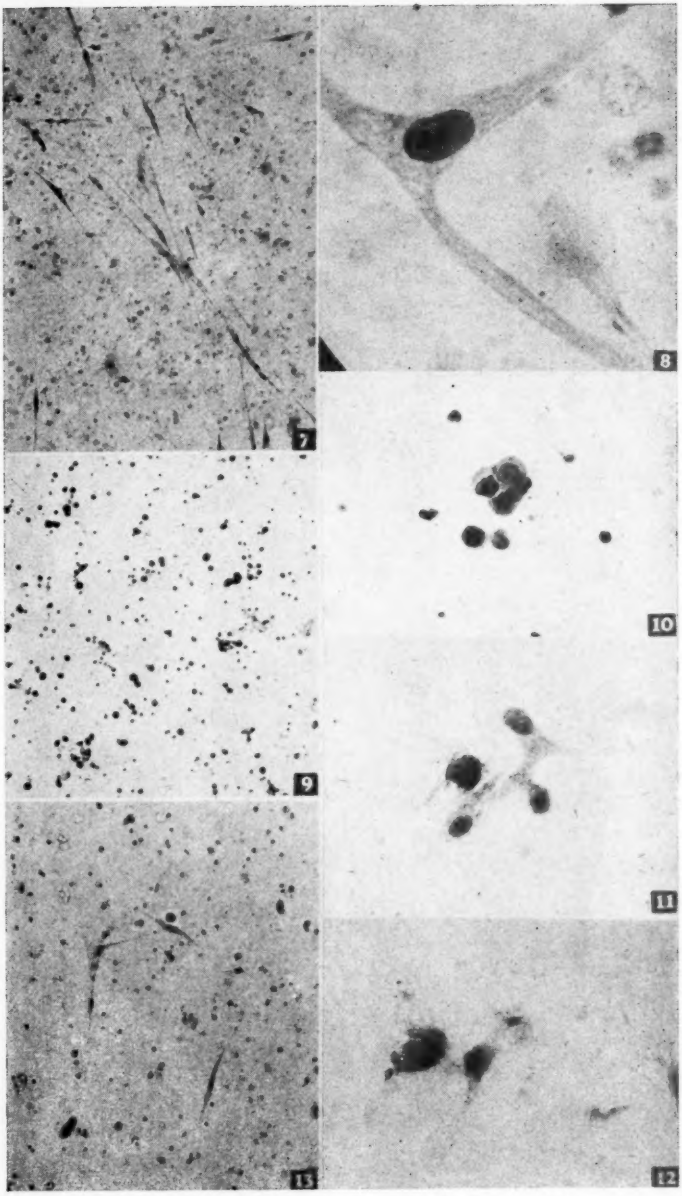
Since malignant cells are present on the walls of the peritoneum in cases of proven neoplastic peritonitis, such elements may be expected in the fluid. The ascitic fluid of Patient C which came to us diagnosed as intra- and extracystic papillary cystadenoma of the ovary with widespread peritoneal metastases, provided such a condition. The fluid was dark in color, almost brown, with a specific gravity of 1.026. A heavy cellular content was obvious grossly as sediment in the bottles before centrifugation. The usual cultures were prepared. At the initial phase the appearance of the cultures was striking. Instead of an even suspension of cells, large and small aggregations were seen. These had a definite cellular architecture and represented the broken ends of papillary structures (Fig. 14). A definite halo could be seen around these solid bits of

---

Figures 7 and 8. Patient A. Figs. 9–13. Hanging drop cultures of cells from ascitic fluid from Patient B. Hematoxylin-Eosin-Azure.

Figure 7. Large spindle cells at 200 hours. 100X. Fig. 8. Same as Fig. 7. 500X.

Figure 9. Initial stage. 100X. Fig. 10. Same as Fig. 9. 700X. Figs. 11 and 12. Polyblasts at 15 hours. 700X. Fig. 13. Large spindle cells at 190 hours. 100X.


7
8
9
10
11
12
13

tissue suggesting that they contained secreting cells. By 12 hours the appearance began to change. The solid structure began to break down and a cystic formation became evident (Figs. 15 and 16). These cysts were unilocular and multilocular. Marked halo formation was evident and the supporting plasma clot lost its typical neutral or slightly basophilic property and stained a deep magenta with an eosinazure stain. By 60 hours sheet-like outgrowths of epithelial cells from the margins of these cysts were commonly seen (Fig. 17). At this stage the sheets were usually one cell thick and frequently had tongue-like processes. The cystic cavities were now larger and contained a very faintly stained homogenous substance when treated only with Delafield's hematoxylin. At 84 hours of growth the cultures had reached their peak of differentiation. The epithelial sheets were much larger and frequently 3 to 4 cells thick near the margin of the cyst itself. Mitotic figures were not seen. In the process of forming the epithelial sheets, the cysts appeared to have flattened out gradually and to have contributed to the enlarging migrating mass. Eventually only slight evidence remained of the location of the original cyst (Figs. 18 and 19). Twenty-four hours later the epithelial sheets began to show degeneration and nuclear fragments were evident. This degeneration continued until at 180 hours of incubation complete disorganization and loss of architecture had occurred.

It is our belief that the events seen in these cultures are analogous to the process of implantation upon the walls of the peritoneal cavity. At the initial stage only solid bits of tissue were seen, but when this tissue was provided with a

---

Figures 14–17. Hanging drop cultures of cells from ascitic fluid from Patient C. Delafield's hematoxylin.

Figure 14. Initial stage, papilliform structures. 290X. Fig. 15. Early stage in cyst formation. 280X. Fig. 16. Photograph focused at level of equator of cyst. Note opalescence of cyst fluid. 300X. Fig. 17. Epithelial sheet formation from base of reorganizing cyst. 290X.

14
16

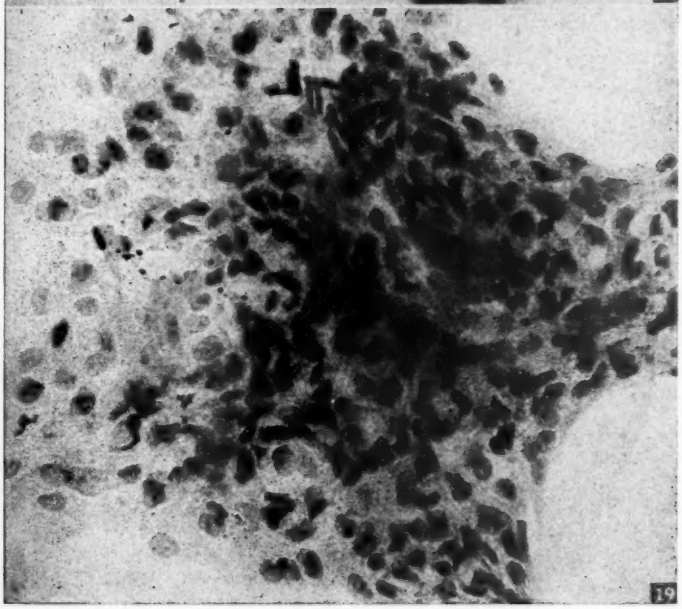
foothold, in this case the plasma clot, it immediately began to form cysts and to migrate out into the solid medium provided.

A second case presenting definitely abnormal cell types was studied. Patient D had a pathological diagnosis of adenocarcinoma of the ovary with metastasis to the peritoneum and to the opposite ovary. The fluid was straw colored with a specific gravity of 1.024. The cellular content was not as heavy as in Patient C. Cells were set up in roller tubes and in hanging drop cultures. Initially free cells were present but more prominent were collections in a plaque formation (Fig. 20). Again these groups seemed to have a definite organization and did not represent merely cellular agglutinations. This should be contrasted to Patient C who showed initially papilliform architectural formations, whereas Patient D presented plaques of 3 to 4 cells in thickness. It was also noted that many of the cells were damaged at the time the cultures were set up. In addition to cell plaques there were also small groups of cells with very densely staining nuclei. These groups were of importance as will be seen later. In the 15 hour cultures many free cells were seen, frequently with large vacuoles in the cytoplasm suggesting intracellular secretory activity. An occasional small cystic structure was noted comprised of cells similar to the vacuolated cells just mentioned. These cysts were very thin walled and fragile. After 39 hours of growth, the cystic structures were more common and larger than previously seen. Many were multilocular with a marked zone of liquefication of the supporting medium around the cyst (Fig. 21). A three-dimensional reconstruction of a well differentiated multilocular cyst is represented in Figure 22. The growth was then progressive until at 110 hours the cultures seemed to reach their peak of differentiation. At this age many cystic structures were present, showing marked variation in size. Some were so large and thin walled

---

Figures 18 and 19. Hanging drop cultures of Patient C. Delafield's hematoxylin. Showing progressive migration of epithelial sheets and disorganization of cystic architecture. 290X.

18



19

that they had collapsed upon fixation. The cysts present at this age were more numerous than could be accounted for on the basis of the number of large plaques seen initially. Therefore some must have developed from the small groups of cells noted at the outset. Some of the cysts appeared to contain no more than 4 to 5 cells and these may have resulted from the proliferation of a single cell. Coman (1943) has observed with similar technique the development of epithelial colonies from one cell. However, it seems curious that mitoses were not found. The cavities of the cysts were perfectly clear. Around the cysts the zone of liquefication persisted and frequently within this liquefied zone round cells were present but these were always degenerative. Such elements were not present in the early stages and therefore might suggest migration of epithelial cells from the cyst margin. Since cells in tissue culture will not multiply in a fluid medium the zone of liquefication may have been a barrier to migration. Free cells were still present in the cultures, many with foamy cytoplasm and many of a large signet-ring shape. Again this suggests intracellular secretory activity, although halos were not present around single cells. After 110 hours the cultures began to disintegrate slowly. Occasional cysts were still present up to 232 hours but by 280 hours universal disintegration was present.

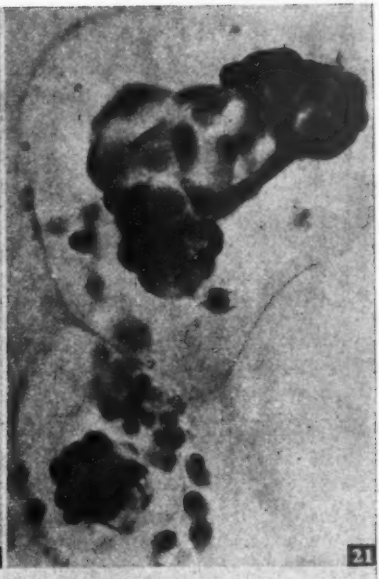
Observations of living cells in roller tube cultures prepared and maintained as described in the first part of this paper demonstrated not only that cells from ascitic fluid of our several subjects became easily established in vitro, but that they could be maintained for at least three months. Since the cellular sediments of our specimens were not adherent in a solid clot but were freely distributed it was possible to set

---

Figures 20–22. Hanging drop cultures of cells from ascitic fluid from Patient D. Hematoxylin-Eosin-Azure.

Figure 20. Initial plaque formation. 290X. Fig. 21. Early cyst formation at 39 hours. Note zone of liquefication around cyst. 290X. Fig. 22. Reconstruction of a multilocular cyst at 64 hours (drawn by Miss Eva Maria Schubart). 330X.

20


21

22

up cultures with known numbers of cells. Figure 23 shows the very uniform distribution of spindle-cells which developed within 48 hours from round cells obtained from Patient A. Long time observation of these cultures, however, did not indicate active multiplication of cells although they appeared perfectly healthy. Further investigation is desirable in order to explore this method for quantitative studies. Results from Patient B were essentially the same as those obtained for Patient A.

Thin-walled fluid-filled cysts of the type seen in hanging drops from Patient C were not found in corresponding roller tubes. Dense clusters comparable to growth shown in Figure 18 were regularly observed (Fig. 24). From such clusters epithelial sheets developed with diameters of 5 millimeters. Individual cells at the periphery are shown in Figure 29. The characteristic appearance of living cells in roller tube cultures from Patient D are shown in Figures 25, 26, 27 and 28. Comparative studies with cells in tissue cultures of other abdominal carcinomas have not been made.

While no systematic study was undertaken regarding the viability of peritoneal cells good growth was obtained from ascitic fluid of Patient C after storage in the ice box at 5° C. for three weeks.

Cultures of Patients C and D contained definitely abnormal cell types and not only did they differ from those of Patients A and B but, as is seen in cultures of rat tumors described by Warren H. Lewis (1936), cultures of various malignancies show special cytological characteristics. Patient D contained

---

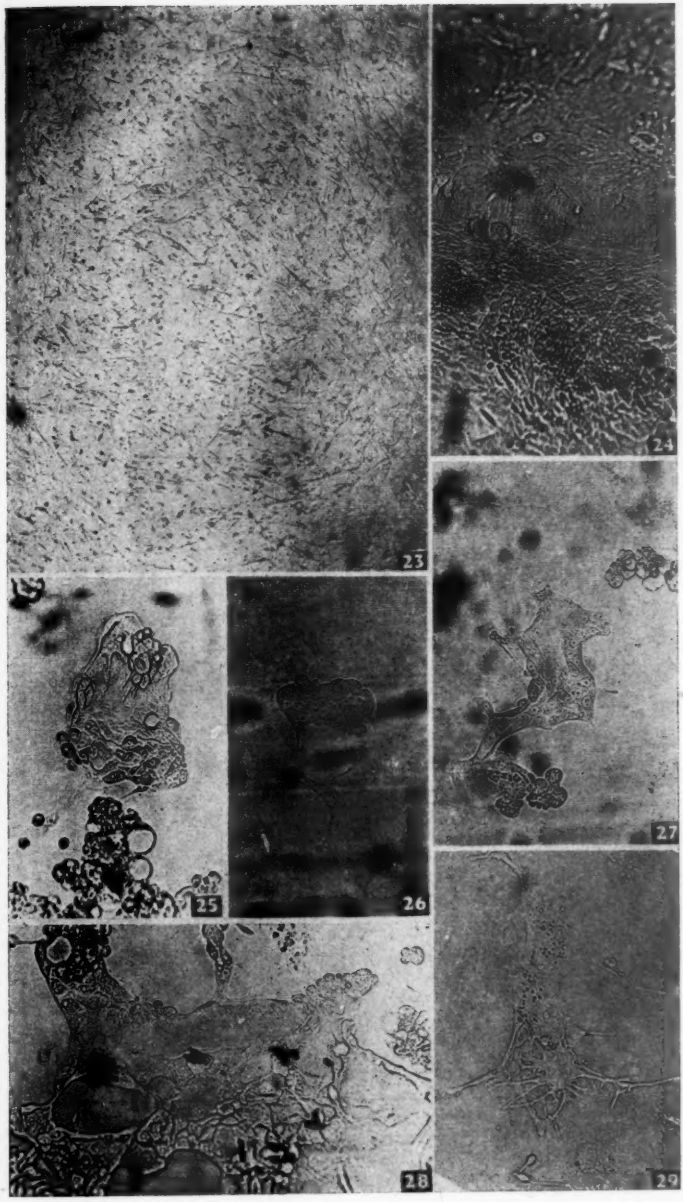
Figures 23–29. Living cells from ascitic fluid through walls of roller tubes.

Figure 23. Patient A, showing uniform distribution of spindle cells after four days in culture. 50X.

Figure 24. Patient C, showing dense clusters of epithelial cells after three weeks of growth. 290X.

Figure 25–28. Patient D, showing epithelial cell formations seen after three weeks of growth. 290X.

Figure 29. Epithelial cell type seen in Patient C. 435X.


23
24
25
26
27
28
29

(1) clumps and plaques of epithelial cells instead of papilliform structures, (2) stained normally with hematoxylin eosin-azure, (3) did not show epithelial sheets migrating from the cysts but a marked zone of liquefication, (4) the cyst walls seemed thinner and the intracystic contents were clear, whereas the intracystic material of Patient C presented a homogenous, cloudy appearance. Their striking points of similarity were the formation of cysts, evidence of secretory activity, epithelial character, similar rates of growth and the absence of spindle-cells.

In summary, we should like to compare Patients A and B with Patients C and D. The former two show very similar cell types, starting with polymorphonuclear leucocytes, lymphocytes, monocytes, polyblasts, and mesothelial cells and ending with pure cultures of fibroblasts. In contrast, C and D resemble each other, starting with formed epithelial tissue bits which developed into well formed cysts, suggesting a definite tendency to gland formation which is probably correlated with their pathological diagnoses. It was surprising that C and D showed no fibroblastic tendencies. It is certain that these patients contained free malignant elements in their ascitic fluid.

In conclusion, we would like to point out that this method of examination, with adequate study, may become a valuable diagnostic aid. It certainly affords a very nice instrument for experimental study of neoplastic cells in the living and fixed states.

REFERENCES

Coman, D. R.: Human neoplasms in tissue culture. II. Observations upon cells derived from peritoneal and pleural effusions. Cancer Res., 3:526–530. 1943.

Downey, H. (Ed.): Handbook of Hematology, Vol. II, Paul B. Hoeber, Inc., New York, pp. 1471–1551, 1938.

Lewis, W. H.: Malignant Cells, The Harvey Lectures, pp. 214–234, 1935–1936.

Lewis, W. H.: Some cultural and cytological characteristics of normal and malignant cells in vitro, Arch. f. expt. Zellforsch., 13: 8–26, 1939.

Maximow, A. A.: Über das Mesothelium (Deckzellen der serösen Häute) und die Zellen der serösen Exudate, Arch. f. expt. Zellforsch., 4: 1, 1–42, 1927.

Maximow, A. A., and Bloom, W.: A Textbook of Histology, (fourth edition), W. B. Saunders Co., Philadelphia and London, 1943.

Wagoner, G., and Custer, R. P.: Handbook of Experimental Pathology, C. C. Thomas, Baltimore, 1932.

Weitzmann, G.: Die Züchtung von Kulturen aus menschlichen Pleura- und Peritonealergüssen, Arch. f. expt. Zellforsch., 22: 414–421, 1938.

THE DIAGNOSIS AND MANAGEMENT OF DIABETIC NEUROPATHY*

RAYMOND GREGORY AND E. L. LINDLEY

Recent experience with a number of pertinent cases has convinced us that established knowledge concerning diabetes mellitus as a cause of neurological disorders is not sufficiently disseminated. This is true not only so far as this information serves as a basis for the proper management of diabetics, but also because of its importance in the differential diagnosis of neurological syndromes.

It is the purpose of this paper to review some of the essential literature and to report illustrative cases.

HISTORICAL

Various types of neurologic changes have long been recognized in diabetic patients. French investigators were among the first to emphasize these changes. Marchal de Calvi in 1864 noted sciatic pains and peripheral areas of anesthesia. In 1881, Bouchard observed the frequent absence of tendon reflexes in this disorder. Althaus in 1885, described the clinical symptoms of diabetic neurologic disorders and in certain cases noted the similarity to tabes dorsalis. Pavy and Leval-Picquechef made significant contributions in the same year. The former noted lightning pains, extreme hyperesthesia of the skin and the intensification of the symptoms at night. The latter introduced the term "pseudo-tabes." Leyden proposed a classification for diabetic neuropathies in 1887 which indicated a sound knowledge of the disorder and one which is useful today. Buzzard observed degeneration of peripheral autonomic nerves; and found that changes in the diet which reduced the glycosuria were beneficial. Charcot described the steppage gait caused by foot drop, and noted the associated vascular lesions in diabetes.

*From the Department of Medicine, The University of Texas School of Medicine and the Medical Service, John Sealy Hospital, Galveston, Texas. Received for publication January 6, 1947.

The neurologic abnormalities associated with diabetes were clearly recognized before the turn of the present century. Indeed it is likely there was an exceptional opportunity to observe the natural history of the disease, uninfluenced by specific therapy, before the advent of insulin.

Jordan has written a comprehensive critical review with extensive literature references covering this subject. He has offered a good working classification of diabetic neuropathies. He has added many carefully studied and critically analyzed case reports to the literature. Rundles has also contributed an excellent review of the subject with a searching analysis of the literature and the addition of 127 well studied cases of his own. Anyone interested in diabetic neuropathy will find it essential and most profitable to refer to these excellent papers.

Incidence

The incidence of neurologic complications of diabetics is not well known. There are enough data however to indicate that it is frequent. Severinghaus reported diminished activities of the reflexes in 57.3% of 75 diabetic patients studied for this condition. Jordan noted the incidence of this complication of the disease in 40% of all diabetic patients as judged by sluggishness or absence of tendon reflexes of the legs. He observed 25 cases of neuritis in 1000 consecutive cases of diabetes observed during the first period of treatment of this disease in these thousand patients. Many more of these diabetics have no doubt developed the neuropathy after the disease had lasted for many years. They were observed for a period of only 2½ years. Bolduan reported that 2% of the population died of diabetes. It can be deduced, therefore, that a large number of people are afflicted with diabetic neuropathy.

Age and Sex Incidence

Rundles found that males constituted 55% and females 45% of the 125 patients whom he studied. This indicates that the male may be slightly more prone to the development of diabetic neuropathy than the female because diabetes

mellitus is more common in women than in men. It is generally recognized that this abnormality is most commonly met with in the diabetic of advancing years. Rundles found that 92 of 125 patients studied were over 40 years of age. This probably is definitely related to the much greater incidence of diabetes in the age groups following 40. It must not be forgotten however that many patients of younger ages are affected with this disorder. Rundles emphasizes that 25% of his patients were less than 40 years of age and eight of his 125 patients were less than 20 years of age. Some of our extreme and dramatic cases have been patients less than 25 years of age who will be discussed in detail later in this paper. Rundles further reported that the average patient with diabetic neuropathy had diabetes slightly over 5 years before the onset of the neuropathy. The range however is from a few months to 25–30 years following the onset of the disease. In some instances, the neuritic symptoms rather than the classic symptoms of diabetes led the patient to first consult medical attention. In all of Rundles' cases, unequivocal symptoms of diabetes accompanied or preceded the symptoms of peripheral nerve disease. He emphasizes that he has not seen a patient where the diabetic neuropathy actually occurred before the appearance of the diabetes mellitus.

Rundles has emphasized that the economic status did not appear to play a role in the incidence of the complication. Fifty-one per cent were welfare type of patients, 34% were of average means and 18% were of superior means which was the average distribution of patients with diabetes and other diseases in the hospital where his observations were made.

Jordan reports 34 patients with the hyperglycemic type of neuropathy 23 of whom were women and 11 of whom were men. The age at the onset varied from 18 to 70 years with an average of 46 years; 9 were less than 40 years of age and 7 were below 30 years of age. He emphasized the common experience that whereas the diabetes was not always severe at the time that the symptoms appeared it was always uncontrolled. This point deserves emphasis. He has reported

45 carefully studied cases in the circulatory and degenerative types of diabetic neuropathy. Two-thirds of the patients in this group were women. The age of the onset of the neuritis varied from 40–70 years with an average of 59; the average duration of the diabetes before the onset of the neuritis was 4½ years. He has careful observations on 120 of the neuritic types of diabetic neuropathy. Of this group 69 or 57.5% were female and 51 or 42.5% were male; the age at the onset of the neuritis varied from 13 to 76 years with an average of 54 years; only 12.5% of the patients were less than 40 years of age and only 5% were less than 30 years, whereas 40% of all persons with diabetes have the disease before the age of 40. The duration of the diabetes at the onset of the neuritic manifestations of diabetic neuropathy varied in Jordan's experience. He reports that 4 patients apparently had neuritic symptoms before the onset of diabetes. This is not in agreement with the observations of Rundles. In 7 others there was a simultaneous onset of the two conditions; the average duration of the diabetes prior to the onset of the neuritic manifestations of neuropathy in his 120 cases was 5.9 years. And in 28 of these cases diabetes had been present for 1 year or less. In 42% of the cases the diabetes was mild, in 43% moderate, and in 15.9% severe.

Distribution of Neurological Manifestations

The portion of the nervous system involved in diabetic neuropathy is extremely varied and widespread. Opinions vary concerning the involvement of the brain, but it is generally held that there may be a characteristic psychiatric picture due to brain involvement. This will be discussed under *Symptoms-Signs*. The cord, particularly the posterior columns are frequently involved. The autonomic nervous system is said to be involved frequently as an explanation of gastro-intestinal symptoms, as well as symptoms involving bladder control. It is significant that the nerves involving the heart have not been described as being affected in diabetic neuropathy. Other evidences of autonomic nervous system disorders are shown by the occurrence of loss of sexual po-

tency in the male. It is the experience of everyone interested in this subject, including our own studies and the written reports, that the greatest incidence is in the extremities. Jordan describes 26 cases of the hyperglycemic type in which 76.5% of the cases showed involvement only of the legs. In the remaining 8 cases or approximately 24%, there was more or less generalized involvement either the arms and legs together or of the entire body. In the circulatory and degenerative types he has described in detail 45 cases. In 18 of these, or 40%, there was involvement only of the legs; in 6 cases the arms and legs alone; 10 of the pupils and legs; and in 2 of the pupils alone; and in the remaining 9 there was generalized involvement. Of the 120 cases of the neuritic type, 81 cases (66.9%) involved the legs alone; 8 or 6.7% the arms alone; in 32 (26.4%) there was general involvement and other special sites.

Rundles review of the literature and his own cases tend to agree with those of Jordan. Of 101 patients, 81% showed absent reflexes of one sort or another in the lower extremities and greatly diminished in another 15 cases, making a total of about 96% in whom there were changes in the lower extremities. He emphasizes that the most reliable sign of diabetic peripheral nerve disease is diminished or absent tendon reflexes. Rundles further reports that there was no significant evidence of clinical nature of primary spinal cord disease or involvement of the lateral or anterior funiculi in his cases. Although such have been thought to occur in diabetic neuropathy, Rundles thinks that good clinical and pathologic evidence is lacking. It is his belief that the symptoms and signs, in general, are those of peripheral nerve involvement with perhaps secondary degenerations of the fasciculi propriae and posterior columns of the spinal cord which in severe cases appear to be anatomically inescapable. Secondary spinal cord tract disease due to peripheral nerve disease seems an unsound concept.

It may be summarized so far as the distribution and site of pathological manifestations are concerned that the ma-

jority of patients with diabetic neuropathy show involvement in the extremities, particularly in the lower extremities.

Classification

The classification of diabetic neuropathy is extremely difficult for a number of reasons. Many of the manifestations of this disorder are acute and disappear with improvement in the metabolic state, whether it be due to control of the diet, or to control of the diet plus insulin. It is obvious that this type of case would not lend itself well to histological study in view of the fact that they always get well and the symptoms disappear. Nothing is known concerning the pathology of the acuter symptoms and signs. Even many of the more chronic symptoms and signs disappear with the passing of weeks, or months and adequate control of the diabetic process. Which means, of course, that these have not been subjected to adequate pathologic study. While changes in the reflexes are often reversible, many of them are not. But even those that are not have seldom been the subject of adequate histological examination. One of the few that is available is the study of Woltman and Wilder who have reported the importance of the arteriosclerotic changes in the intraneural blood vessels as a cause of at least many of the more chronic types of symptoms and signs. Wilder has emphasized, on the basis of the studies of Woltman and Wilder, that marked degenerative changes in nerves may be present with but very few symptoms or signs. It is difficult to consider that many of the very acute symptoms and signs of diabetic neuropathy which are notable for the rapidity with which they improve under adequate management of the diabetic process could be due to degenerative lesions of nerves regardless of whether it is on an arteriosclerotic obliteration of the intraneural arteries or whether it is on the basis of some other degenerative process. In general, one of the features of degenerative changes in nerves is the slowness with which they recover. Little, therefore, is known about the specific pathology of the process in the nerves or in the nervous system. In view of the fact that there is as yet no firmly established

knowledge concerning the etiological mechanism involved, it is equally difficult to give the classification on the basis of etiology or etiologic mechanisms. As has been mentioned before, the very old classification of Leyden into the (1) hyperesthetic or neurlagic, (2) motor or paralytic, (3) ataxic or pseudotabetic forms with peripheral sensory deficiencies, absent tendon reflexes and absent pupillary reflexes indicated a sound knowledge of the processes of diabetic neuropathy which would do credit to a classification of today.

Jordan has used and proposed a very workable classification as follows: (1) hyperglycemic type, which is comprised of cases who show neuritic symptoms, usually without any signs other than tenderness of the nerve or muscles affected. They are associated with an abnormally high sugar content of the blood and they disappear within a few days after the initiation of adequate diabetic treatment and do not recur if the diabetic regimen is followed. (2) The circulatory type which includes those cases which show considerable circulatory deficiency in the legs and in addition, pain, parestesia or signs such as hyporeflexia and hypesthesia. (3) The degenerative group which are characterized by rather mild neuritic symptoms and signs which begin insidiously and tend to progress slowly over a period of years. This group includes such cases as show sluggish pupillary reactions and hyporeflexia. Occasionally the neuropathy involved structures which, by their nature, drew both the patient's and physician's attention to them as in the cases which involved the bladder. (4) The neuritic group which embrace cases in which there are definite manifestations of neuritis usually with relatively severe symptoms or signs such as paralysis. The onset is reasonably acute and improvement takes place only within weeks or months following adequate treatment. Jordan himself believes that in reality his groups 2 and 3, namely the circulatory and the degenerative types, probably are identical and treats them as such.

Wilder is inclined to accept only 2 real categories, namely, the hyperglycemic and the neuritic and thinks that the circu-

latory and degenerative group proposed by Jordan in reality belongs to the neuritic classification.

It is unlikely that the high blood sugar itself is responsible for the neurologic abnormality. The true etiologic factor has probably something to do with the uncontrolled diabetic process. And as long as the hyperglycemic classification is used to emphasize this point of view, it serves a worthwhile purpose, although no one in the field believes that the hyperglycemia *per se* is the responsible mechanism.

Etiology and Pathogenesis

The etiology of the neurologic changes in diabetes has been variously ascribed wholly or partially to the excessive levels of sugar in the blood, cachexia, incomplete combustion of organic acids, vascular disease, dehydration, an unknown toxic substance due to the diabetic condition, acetonuria and deficiency diseases.

The role of diabetes mellitus and its disturbed metabolic state has also been proposed, particularly by Jordan, as a sensitizing mechanism with the precipitating factor served by a number of other agents, both toxic and bacterial in nature. This is speculative. There is no direct evidence to support it. The role of hypercholesteremia which occurs frequently in diabetes has also been considered but no evidence again has been adduced to support any belief that this may be the specific mechanism involved. Widespread arteriosclerosis, including intraneural arteries, has also had many advocates. The fact that most patients with diabetic neuropathy are beyond 40 years of age is frequently, though inadequately, cited as probable support for the contention that the arteriosclerotic process is of great importance. Woltman and Wilder have adduced evidence and have been one of the principle proponents for the arteriosclerotic factor as an etiologic agent of importance. Wilder however is not entirely satisfied with the idea that arteriosclerosis is the principal cause of diabetic neuropathy. He has emphasized that the extent of the degeneration seen in the nerve is by no means proportional to the degree of thickening of the walls of the nutrient arteries.

And Jordan, Randall and Bloor found occasionally marked variation in the lipid content of the nerves which is unexplainable on the basis of other known features of diabetes. Wilder further points out that if arteriosclerosis *per se* were the principal cause, why is this type of neuritis with its characteristic darting pains occurring at night and relieved by exercise, not encountered more frequently in nondiabetic patients with obliterating arteriosclerosis in which the circulation is equally or more impaired.

In discussing the pathogenesis of diabetic neuropathy Rundles' experience confirms in general the literature and the experience of those working in this field, namely that this complication of diabetes makes its appearance only after months or even years of grossly uncontrolled diabetic state. Lack of control, is evidenced by duration, severity of the known manifestations, of the known elevation of blood sugar and urine sugar and the body weight lost, the presence of other complications of the diabetic state, etc. He states that it has long been known that there is no correlation between the severity of diabetes or any one acute complication of diabetes such as coma, acidosis, or ketonuria and the incidence of diabetic neuropathy. Rundles observed that in 56% of his patients, for example, there had never occurred ketonuria even at the highest peak of the diabetic process. He further points out however that there is a striking correlation between diabetic neglect, of a degree permitting the patient to survive in his debilitated state of health without succumbing to the acute complications of diabetes and the ultimate development of neuritic complications. Rundles concludes that information available justifies the opinion that neither occlusive vascular disease nor a primary vitamin B deficiency plays a definite etiological role. His clinical study allows him to further feel that the conclusion is inescapable that diabetic neuropathy is not only truly diabetic in etiology but results from abnormal metabolism of chronically unregulated diabetes.

Jordan, Randall and Bloor have studied the phospholipid cholesterol and cerebroside content of peripheral nerves in patients with diabetes. They have reported that values for

these lipoid substances were considerably lower on the average than those found in normal nerves used for controls in non-diabetic patients. The greater the vascular disease present and the lower the level of the nerve examined, the more marked was the chemical changes. The effect of the severity, duration and control as judged by glycemia and glycosuria of the diabetes was slight or vitiated by other factors. Occasionally marked variations occurred unexplainable on the basis of vascular disease or of the known features of the diabetes. The lipoid abnormality appears in the absence of clinical evidence of neuropathy but the degree of the abnormality increased as the clinical signs of neuropathy progressed.

The present concepts of the pathogenesis of diabetes, particularly as proposed and supported by the work of Stadie who holds that insulin deficiency leads to interference with carbohydrate utilization. When the diabetic, by reason of insulin lack, cannot utilize carbohydrate in an amount adequate to satisfy the metabolic needs protein and fat, the latter especially, is oxidized to meet energy requirements. A considerable fraction of the fat undergoes a preliminary oxidation in the liver, the ketone bodies are then completely oxidized peripherally. The ketones are not abnormal metabolic products accordingly and cannot be considered toxic or harmful to nervous or other tissues. When metabolism of fat exceeds a certain maximum, about 150 gms./day, ketonuria appears. This in turn leads to the development of diabetic acidosis. The clinical studies and reports of Rundles indicate that peripheral nerve disease arises when these metabolic adjustments, consequent to insulin deprivation fail to maintain adequate nutrition. Rundles further postulates that in considering the metabolic origin of diabetic neuropathy, the carbohydrate deprivation may be paramount but the excessive oxidation of fat possibly leading to peripheral demyelimization as shown by the work of Jordan, Randall and Bloor as well as the protein breakdown which occurs cannot be ignored.

It is long established that normally carbohydrate is the principal type of foodstuff utilized in the metabolism of the

nerve tissue (Quastel). On the basis of this, Rundles postulates that it would not be unreasonable to consider as possible that any disease interfering with carbohydrate utilization might lead to neuropathy. Rundles attempts to draw the following analogy between thiamin deficiency, which perhaps is the commonest best understood cause of peripheral nerve disease and diabetic neuropathy. Thiamin deficiency produces a "biochemical lesion" in carbohydrate metabolism wherein there is a failure of pyruvic oxidation (Peters), with the accumulation of this substance and lactic acid in the tissues. If abnormal carbohydrate metabolism, leading in effect to neurone starvation, proves to be a common denominator in different types of peripheral neuropathy, it is likely unimportant as regards the end result if the defect occurs at the 6 carbon stage, as in diabetes mellitus, or at the 3 carbon stage, as in thiamin deficiency.

Role of Vitamin Deficiency

In view of the frequency with which diabetic neuropathy is confused with thiamin deficiency, and with which thiamin is used in the management of diabetic neuropathy, a word concerning the best opinion on this subject seems advisable. Many studies have been made to implicate thiamin deficiency as the cause of the neuropathy associated with diabetes. The deficiency of the diet has been studied by Jordan and Needles. The former investigated the diet in 63 patients with diabetic neuropathy and found only one whose diet appeared to have been deficient. Needles made a very careful study of the antecedent diets of two diabetic patients who developed peripheral nerve disease and found that the thiamin content had been more than ample. Faulty absorption and excessive excretion of thiamin in the urine has been also implicated as a cause of the thiamin deficiency. Robinson, Melnick and Field investigated 6 diabetic neuropathies, 4 of whom had peripheral nerve disease and all 6 of them were found to have normal excretory values of urinary thiamin. Needles has also studied thiamin excretion in 6 diabetics with well substantiated neuropathy. In five of them he found entirely

normal values and in one a value slightly below normal. The work of many other confirmatory authors could be quoted to support the results mentioned above.

Another approach has been made to this by a number of authors including the giving of thiamin supplements to the diet and observing the effects on the neuropathy. Thus Needles treated seven patients with severe characteristic diabetic neuropathy over an average period of 5½ months with daily doses of 10–15 mg or more of thiamin. He concluded that thiamin was of no value therapeutically, and in fact two patients became progressively worse during its administration. Rundles reported that at least six of his patients had been given large doses of thiamin orally and parenterally for several weeks or even months by their home physicians while the diabetes remained uncontrolled. In all cases the neuritic disease became progressively worse. In spite of the observations cited above that signs of avitaminosis in diabetics can be cured by specific vitamin therapy even though the diabetes remains uncontrolled, Rundles reports that he has never seen clinical improvement in diabetic neuropathy by any treatment regimen in the absence of effective diabetic control. Rundles gave thirty-two hospitalized patients 10–20 mg. thiamin orally for 2–3 weeks or longer. They were also given 40–50 mg. of brewer's yeast daily in addition. No definite benefit could be ascribed to the vitamin administration. The addition of 200–400 mg. of nicotinic acid daily to the diet likewise failed to be beneficial. Daily intramuscular injections of 20–50 mg. of thiamin hydrochloride up to such doses as 1000 mg. in 14 days and 1900 mg. in 25 days, in addition to 40–50 mg. of brewer's yeast daily by mouth, failed to modify the neurologic status in several instances, as reported by Rundles.

Joslin, Wilder, Levy and White have also found thiamin and other vitamin fractions ineffective in the therapy of diabetic neuropathy. This has been amply borne out in our own experience over a period of a good many years.

It may be summed up by saying that there is no evidence to support any idea that thiamin deficiency is the etiologic

factor in diabetic neuropathy and there is no good evidence to support the belief that thiamin or any other member of the B fraction is of any value when used therapeutically in the treatment of diabetic neuropathy.

The Role of Insulin

Everyone interested in diabetes has noted a number of cases in whom there is an acute intensification of the neuritic disease shortly after control of the diabetes is undertaken. This is a poorly understood and disturbing phenomenon encountered in the treatment of diabetic patients with associated neuropathy. The authors have observed a patient who was under treatment for diabetic acidosis and who became completely paralyzed in all extremities for the first time about one week following the institution of therapy.

Rundles reported that twenty of 125 patients (16%) with diabetic neuropathy became much worse some two or more weeks after diabetic treatment was started. Caravati has described this phenomenon as "insulin neuritis."

Although numerous workers have observed this phenomenon after the institution of insulin therapy, it may be stated with considerable assurance that insulin is probably not the cause in view of the fact that even though these cases do occur following institution of insulin therapy, they get better with the continuation of the insulin administration. Rundles has emphasized that he sees no reason whatsoever to incriminate the use of insulin in the etiology of diabetic neuropathy. With this viewpoint the authors are in agreement. Jordan states that he hesitates to attribute any of these acute intensifications of the neuropathic process to insulin administration and yet he has no better explanation for the process.

Pathology

The pathological process underlying the acute manifestations of diabetic neuropathy, particularly those that clear up within a few days to several weeks after the institution of adequate therapy, is unknown for the obvious reason that there are no autopsy or surgical specimens on this type of

individual available for study. The rapidity with which the symptoms and signs develop and are relieved by adequate treatment naturally suggest the likelihood that they are in some way related to functional disturbance of nerves rather than any organic changes in the nerve fibers or nerve cells themselves.

We have no pathological studies on the nerves in any of our cases. The literature shows that changes have been noted in the spinal cord by nine authors (Minor) in the cord and nerves by eight (Leichtentritt), in the peripheral nerves by eight (Pryce) and in the posterior roots by three (Williamson). In Woltman and Wilder's excellent review of this subject they described 42 cases gathered from the literature in 24 of which there was degeneration of the peripheral nerves, 16 showed degeneration of the funiculi, in 8 changes were noted in the anterior horns and in 4 there was intramedullary degeneration of the posterior roots. Williamson described parenchymatous neuritis with myelin degeneration and changes in the axis cylinder in isolated nerve fibers. He also noted normal tissue proximal to markedly diseased nerve tissue and from this concluded that the changes in the nerve were not secondary to the changes in the spinal cord.

Many authors have commented upon the vascular disease of the intraneural arteries. Woltman and Wilder's studies particularly emphasize this. They noticed slight changes in the vessels of the cord and marked changes in those of the nerves so far as sclerosis of the intraneural vessels were concerned and concluded that this arteriosclerosis was a very important etiological agent. In addition to these changes they also found patchy degeneration of nerves of myelin degeneration, at times infiltration by lymphocytes, polymorphoneuclears. The lesions are more marked in the peripheral than in the proximal part of the nerve. Warren studied the pathological process of many diabetic patients, a large number of which were nerve specimens from amputated legs. His findings showed that the pathological process in the nerves was not very conspicuous but the small peripheral branches of

the nerves were not studied. Sclerosis of the intraneural arterioles and occasionally myelin degeneration were observed.

As will be noted in the subsequent discussion of symptoms and signs of diabetic neuropathy there is much evidence to indicate that the autonomic nervous system is involved. But a precise study of the exact pathology in the autonomic nervous system has apparently not been made insofar as our knowledge is concerned.

Symptoms and Signs

The symptoms and signs of diabetic neuropathy are extremely varied depending upon the portion of the nervous system which is involved. They will therefore be discussed in terms of regional involvement.

1. *Brain:* Psychic symptoms of many different sorts have been described in patients with diabetic neuropathy. It is difficult to evaluate many of these symptoms because there is such a high coincidence of hypertension and arteriosclerosis which produce their usual symptoms in the brain. It is also difficult to be sure that many of the symptoms attributed to diabetic neuropathy in the brain may not be ordinary psychoneurotic manifestations of the particular individual which might develop from the stress and tension of adapting to any chronic disease.

Many authors describe a peculiar psychic state encountered in patients with diabetic neuropathy and emphasize the inordinate depression and emotional instability that occurs. We have seen several of these in our own experience. It is impossible to be sure however that these psychic manifestations are not disorders of personality produced by failure to adapt to the management of a chronic process rather than to the diabetic effect *per se* on the brain.

2. *Cranial Nerves:* Optic neuritis or retrobulbar neuritis due to diabetes mellitus has been described by several authors. Jordan found two diabetic patients with neuritis of the optic nerve with no obvious cause unless it was diabetes. One other patient with diabetes developed an optic neuritis fol-

lowing removal of the teeth. Jordan also describes the case of a thirteen year old boy who had an optic neuritis without any apparent cause except diabetes. In both of these patients recovery occurred with no treatment other than the usual diabetic regime.

Many authors have described paresis or paralysis of the external ocular muscles. Some of these cases are acute in onset and clear up rapidly with proper management of the diabetic state very similar to the paralysis of muscles in other parts of the body. Root reported five cases of paralysis of external ocular muscles in diabetics in three of which cases there was evidence of diabetic neuropathy in other parts of the body. Jordan reported the case of a 64 year old man with diabetes who developed paralysis of both superior rectus muscles and the left external rectus muscle acutely. Recovery was complete with control of the diabetes.

Various types of involvement of the auditory nerve and facial nerve have also been described by a number of authors. Jordan reported the case of one patient who had a temporary paralysis of the superior laryngeal nerve which he attributed to diabetic neuropathy.

Abnormal pupillary reactions are extremely common in diabetic neuropathy according to many authors. Rundles reported that 25% of his cases of diabetic neuropathy showed abnormal pupillary reactions. The principal findings in his cases were a miosis and poor reaction to light with a better reaction to accommodation. Irregular and unequal pupils have also been observed. Rundles also reported two cases of Argyll Robertson pupil in two patients in whom there was no evidence of syphilis. He also observed paradoxical pupillary reaction of the pupils to light in one case; and that the abnormal reaction to light improved with the treatment of diabetes in many instances. Many other authors have reported abnormal pupillary reactions, including the Argyll Robertson type, associated with diabetes mellitus and apparently not due to any other cause.

3. *Spinal Cord:* Many authors have described lesions in the posterior column of the spinal cord. In view of this one

would expect to find ataxia frequently. This appears generally not to be the case. In one series reported by Jordan ataxia was found only four times. In each instance there were diseases such as syphilis, combined system disease and in two there was a history of the consumption of alcohol. Rundles interprets the changes in the posterior columns of the spinal cord as being secondary to changes in the peripheral nerves. With this line of reasoning it might be easy to understand how there would be secondary degenerative changes in the posterior roots and posterior root ganglia, but difficult to see how these could secondarily result in changes in the posterior column.

4. *Autonomic Nervous System:* The symptoms and signs resulting from the changes in the autonomic nervous system naturally depend upon the organs which are involved. These abnormalities will be discussed under (a) Cardiovascular; (b) Gastrointestinal; (c) manifestations of autonomic dysfunction in the periphery.

(a) *Cardiovascular manifestations of autonomic dysfunction.* Orthostatic hypotension has been recognized for a long time to be associated with various disturbances of the function of the sympathetic nervous system. This condition has been found to be associated with tabes dorsalis, unclassified diseases of the central nervous system, and sequelae of encephalitis among others. It is therefore not too surprising to find that Rundles observed this defective orthostatic regulation of the blood pressure in eight patients with diabetic neuropathy. Whether this was due to peripheral neuropathy or cord disease cannot be determined at this time. In view of the facts that are known concerning its cause in other types of neurologic disturbances it may be assumed that the defect was most likely due to abnormalities of the spinal cord rather than to disease of the peripheral nerve. The lack of reflex cardiac acceleration which is described as characteristic of orthostatic hypotension in general was not observed in the patients studied by Rundles. Five of these eight patients had typical symptoms of orthostatic circulatory insufficiency. Three of them did not have definite symptoms but had excessive falls in blood

pressure with postural change and also exhibited orthostatic tachycardia.

(b) *Gastrointestinal Symptoms.* Rundles has reported striking abnormalities in the form of severe constipation, chronic diarrhea, anorexia and nausea which often accompany the development of diabetic neuropathy. After he had eliminated symptoms due to other possible gastrointestinal diseases by the customary diagnostic methods of X-ray, sigmoidoscopy, stool examinations, etc., 76 of 125 patients had such symptoms. He emphasized that constipation developed in 53 of these patients with the development of neuritic symptoms. In 22 of them the condition was so refractory as to require the persistent use of enemas to assure bowel movements oftener than every 4–6 days to prevent fecal impaction. He noted chronic diarrhea with 4–6 liquid stools not attributable to other causes, and one patient died in the hospital of a diffuse exsanguinating gastrointestinal hemorrhage one week after severe diabetic acidosis was treated. Stool examinations in the hospital failed to reveal excess fat, undigested meat fibers, ova or parasites. A "diabetic diarrhea" has received some attention for many years. Bowen and Aaron associated achlorhydria with diarrhea of ten diabetics. Bargen, Bollman and Kepler compared the diarrhea of diabetics with the steatorrhea of pancreatic insufficiency and found that only the latter condition responded to the oral administration of pancreatic extracts. Marble also described diarrhea as a grave as well as a distressing complication of diabetes and suggested the possibility of a neurogenic origin. White noted nocturnal diarrhea frequently particularly as an accompaniment of neuritis in young diabetics. Rundles reported findings of two patients with these symptoms who died and were reported pathologically to have degenerative changes in the nerve trunk of the esophageal plexus and about the celiac ganglia.[9] He emphasizes that there is no clinical evidence to suggest that neuropathy follows diarrhea in sequence, as in some of the deficiency diseases, although the presence of diarrhea and even more the occasionally associated anorexia and nausea makes the diabetic status exceedingly difficult to treat.

(c) *Peripheral autonomic nerve disease.* The involvement of autonomic portions of peripheral nerve in different types of neuropathy has been recognized by several writers (Guttman, Wilkins and Kolb, and Shumacker).

Rundles has given particular attention to this phase of diabetic neuropathy and has uncovered evidences of it in 35 patients whom he personally studied. After excluding patients with cardiac disease, nephritis, low serum proteins, varicose veins, etc., he has found 26 patients who had dependent edema of the lower extremities appearing during the course of the diabetic neuropathy. This type of edema is known to occur in many different types of peripheral neuropathy. It is presumably due to involvement of the vasomotor nerves (Pryce and Buzzard).

Thirteen patients noted decreased or absent sweating over the feet and legs usually in areas of sensory disturbance. Rundles reported objective confirmation of this abnormality in eleven patients by the use of the starch-iodine sweating test. He also reported two patients who had localized areas of painful hyperesthesia in which there occurred profuse sweating at times when the skin elsewhere was dry. He also observed drenching night sweats in six patients who were afebrile who were demonstrated to be free of all infections. He has suggested that these observations imply impaired temperature regulating mechanisms perhaps due to imperfect control of the sweat glands and vasomotor adjustment of cutaneous blood flow. Rundles also observed four patients with chronic neuropathy in whom the skin over the extremities became shiny and atrophic; and one patient in whom a dry and fissured skin occurred apparently due to decreased function of the sebaceous glands. He has also found evidence of loss of vasomotor and pilomotor control in a number of his patients.

5. *Neuropathy Involving Bladder Control:* Reports in the literature of genito-urinary and sphincter disturbances in diabetic patients with or without other symptoms or signs of diabetic neuropathy are numerous. McKittrick and Root reported three diabetic patients with chronically distended, para-

lyzed bladders in whom no generalized or local cause was found. Jordan and Crabtree studied seven patients with distended bladders and large urinary residuals. At least six of these patients showed other neuritic symptoms and evidence of organic nerve disease and Jordan and Crabtree attributed the bladder paralysis to diabetic neuropathy. Jordan has subsequently added five additional cases. The experience of Jordan would lead one to believe that the prognosis in these bladder disturbances is generally poor. Rudy and Muellner gave a detailed study of eleven patients having diabetic bladder paralyses, in ten of whom there was evidence of neuritic disease elsewhere. They performed repeated cystometric examinations and were able to show gradual improvement during the course of treatment.

Of the 125 diabetic patients with diabetic neuropathy reported by Rundles, thirty-two (25%) had genito-urinary and sphincter disturbances. There was complete lack of sexual potency in nineteen patients (27.5% of all males) and this preceded any other symptom of peripheral nerve disorder. Urinary incontinence was a complaint of ten patients. Five of these and two others, also, had fecal incontinence.

The principal symptoms which Rundles noted were as follows: difficulty in urination, hesitancy, a slow, weak stream, or acute urinary retention that led to the discovery of an atonic type of bladder paralysis in eighteen patients, fifteen of whom were males. Thirteen of these had cystometric examinations which showed impaired or absent sense of filling, very low expulsive force, and an increased bladder capacity. The residual urine varied from nothing to over 300 cc. Uninhibited bladder contractions did not occur. Special investigations including sounding and endoscopy were carried out routinely in order that vesical neck and urethral obstructions might not be overlooked before concluding that the atonic bladder paralysis was neurogenic in origin. Fifteen of the eighteen patients with bladder paralysis had impotence or defective sphincter control. Two patients with atonic bladders had impotence and both urinary and fecal incontinence. Of the thirty-two patients with genito-urinary and sphincter dis-

turbances, twenty-five (78%) had either severe chronic constipation developing with the neuropathy (12 cases) or some type of chronic diarrhea (13 cases).

In two of the patients described by Rundles there were atonic bladders and exquisite cutaneous hyperesthesia in the pudendal area. The bladder paralysis of diabetic neuropathy has been classified by Nesbit and Gordon as atonic neurogenic paralysis such as occurs in tabes dorsalis, pernicious anemia with peripheral nerve and posterior column disease. In the diabetics studied by Rundles an impaired or absence of bladder filling was the only constant sensory defect found by cystometric examinations. Whether the bladder syndrome is wholly sensory in origin or whether there is decreased motor activity, too, is difficult to determine according to Rundles. Loss of ejaculation similar to that found following lumbar sympathectomies was described by Rundles to have occurred in a man with diabetes. This patient had recovered eighteen years previously from an evidently severe diabetic neuropathy. In this patient there was complete absence of ejaculation which followed the acute episode although he retained a nearly normal capacity to produce an erection. Careful examination showed dependent rubor of the feet which subsided with elevation of the legs, an abnormal orthostatic fall in blood pressure, and diminished sweating below the knees with absent sweating over the hands and feet. Cystometric examination showed a bladder capacity of over 650 cc., first desire to void at 475 cc., residual urine 150 cc., and sense of filling absent but sense of hot and cold present. The residual sympathetic nerve degeneration was thought to account for the loss of ejaculatory power. There was almost invariable association of complete impotence with atonic bladder paralysis in males. There was an exception to this in only one case. Rundles cites this as evidence that impotence in patients with diabetic neuropathy is likely to be a symptom of organic nerve disease.

6. *Neuropathy in the Extremities:* By far the greatest number of patients suffering from diabetic neuropathy have manifestations in the extremities. Even though there are signs or symptoms of the abnormality in other portions of the body,

they are usually associated with abnormalities in the upper or lower extremities. The lower extremity predominates by a wide margin. As has been noted elsewhere, Jordan found that the legs were involved in 66.9%, arms 6.7%, general involvement and other special sites 26.4%. This predominance of involvement of the extremities, particularly the legs, has been borne out in all reports.

Rundles reported that muscular cramps and aching were frequently early symptoms and muscular weakness, especially of the legs, was almost uniformly present. Four of his cases manifested an extreme degree of weakness in that they were unable to go upstairs at home for weeks except by crawling on the hands and knees. In two other patients muscular exhaustion was so great as to enable them to walk only a few feet. Severe neuropathy developed acutely in another patient one week following diabetic coma and it manifested itself in the form of complete flaccid paralysis, bilateral wrist and foot drop. The patient was unable to do anything for herself for 3 or 4 months before slow improvement began. Muscle tenderness aggravated by use and exertion was present in one-third of his cases and often persisted for months even after treatment was begun. Other authors, particularly Jordan, have emphasized that exercise often relieves the various symptoms of muscle cramps and tenderness which have been precipitated by rest in bed. Numbness, tingling, and paresthesias such as "cold" feelings, aching and burning pains, etc., were usually prominent among Rundles' patients' complaints. Severe "shooting pains" in deep and superficial tissues were noted in more than one-fourth of his patients. Cramps, aches, and pains were characteristically worse upon exposure to cold and at night. The accentuation of the subjective symptoms of the abnormality in the lower extremities at night has been commented upon by most people interested in this field. At times the touch of bedclothes is often unbearable. Peripheral areas of anesthesia and unsteadiness of gait were found but less commonly. Foot drop was noted by a few ambulatory patients and once occurred practically as an isolated symptom in a case reported by Rundles. Rundles further reports that

distal involvement of the extremities showed the peroneal nerve to be more vulnerable than the tibial, and the ulnar more vulnerable than the radial or median nerves. Rundles also reports that occasionally symptoms occurred predominantly in the pudendal, femoral, or intercostal nerve areas, or asymmetrically distributed in the extremities.

The symptoms of diabetic neuropathy were frequently intermittent or recurrent with gradual increase in intensity over a long period of time before assuming serious proportions. With uncontrolled diabetes the tendency was toward increasing severity and persistence of symptoms.

In general diminished or absent tendon reflexes is the commonest neurological abnormality. The Achilles tendon reflexes were found to be absent in 101 (81%) of Rundles' cases and greatly diminished in another fifteen cases. The patellar reflexes were absent on one or both sides in seventy cases (56%) and greatly diminished in twenty-nine more. Diminished or absent biceps and triceps reflexes were found in one-third of the cases. In several cases there was complete areflexia. The cutaneous sensibilities were blunted peripherally in about half of the patients. In this group areas of hyperesthesia and plantar dysthesia were commonly found, and in nine patients areas of complete anesthesia occurred. Pronounced muscle tenderness was present in 46 cases (36.8%) (Rundles). Deep pain sensibility was unusually altered except in patients with excessive muscular tenderness in whom increased sensibility was the rule. The vibratory sense at the ankles was diminished in 37 patients and absent in 20. In seven patients the vibratory sense was lost below the pelvis. Complete foot drop was present in ten cases. The plantar responses were always in the direction of flexion. Clonus, exaggerated tendon reflexes, and spasticity was never encountered.

7. *Miscellaneous Findings:* Mild degrees of elevation of temperature and mild degrees of elevation of the leukocyte count unattributable to any other cause has been reported by Jordan.

8. *Laboratory Findings:* Other than the hyperglycemia and glycosuria and occasionally the findings of acetonuria, etc., which may characterize the diabetic state, there is but one laboratory finding which needs emphasis. That is the findings in the spinal fluid. The abnormal findings are, in addition to the elevation in the glucose content, concerned with the changes in the protein content of the spinal fluid.

Many observers have noted this change in the protein content which may be, and usually is, relatively slight but on other occasions, it may be marked. Of thirty-nine examinations of spinal fluids made in thirty-one patients by Rundles, ten of the fluids were normal. Thirteen had proteins ranging from 48–80 mg. per cent and three patients showed values as high as 268, 364, and 426 mg. per cent.

In view of the muscular weakness and other changes in the reflexes and sensory findings, caution must be observed in order that diabetic neuropathy not be confused with the Gullian-Barré syndrome which also shows changes of neurologic nature together with an increase in the protein content of the spinal fluid. In general, a distinction may be made, at least in the acute stages, by the fact that there may be some increase in the cell count in the Gullian-Barré syndrome although the increase in the cell content is very disproportionately low compared to the increase in the protein content.

Treatment

The treatment of diabetic neuropathy is in essence a very simple matter. It consists in the rigid control of the abnormal metabolic process with proper application of dietary and insulin management. This must be maintained on a very adequate level of management for at least weeks, and months in many instances, to obtain the relief which these patients can be afforded with the proper degree of management of their abnormal metabolic state.

It may be further emphasized that many of the older patients, particularly those complicated by hypertensive disease may show such abnormally high threshold values for sugar that the urine sugar content is a poor criterion for successful

therapy. These patients should be managed to the point where they have fasting blood sugars ranging from 80–100 mg. per cent or very little above these values.

The prevention of diabetic neuropathy is one of the many reasons for acceptance of the desirability of reasonably adequate control of the diabetic process. It is not merely enough to carelessly manage the diabetic only to the point of avoiding acidosis. There are sufficient instances in the experiences of everyone interested in the treatment of this disease to emphasize that it is the uncontrolled diabetic who develops the neuropathy.

Symptomatic treatment over the weeks or months during which rigid control of the diabetic process is required to achieve lasting results in the symptoms and signs are of some value. These measures include the necessity for considering the fact that in many cases, the diabetic neuropathy is an exceedingly painful affliction and one which often persists over a period of many weeks or months. Symptomatic treatment is therefore of major importance. The analgesic effects of salicylates, the use of cradles to prevent contact with the bedclothes, judicious use of warm and cool baths are helpful measures at different times. Opiates are sometimes required even though at the risk of possible addiction. Severe refractory pain in well localized or areas restricted to the territory of a single peripheral nerve have been treated in some instances by local anesthesia and nerve crushes or nerve sections. At times this has been helpful.

Prognosis

As has often been noted (Buzzard, Jordan, Brain, Joslin, Root, White and Marble, Angle, Rudy, and Major), the prognosis in diabetic neuropathy is usually good. This is particularly true in the younger age groups in whom there are no complications such as perforating ulcers, neurotrophic joint destruction, etc. It is essential however that adequate treatment be provided reasonably early before permanent nerve damage has occurred. Satisfactory diabetic control must be maintained over an adequate length of time. The time re-

quired for recovery varies according to the severity of the neuritic disease. In mild cases a few weeks will be required, in moderately severe cases 6–12 months and in severe cases 1–2 years. Residual neurological signs will often persist indefinitely. But the symptoms in general will be benefited, if not completely relieved, by good management of the diabetes for an adequate period of time.

CASE REPORTS

A series of 9 case reports is presented to illustrate the discussion of diabetic neuropathy:

Case No. 1803: A white female, 47 years of age, sought treatment for weakness, polydipsia, polyphagia and polyuria, six months duration, and numbness, tingling, aching pains and tenderness of legs and feet, three months duration. Tenderness was so severe that the patient could not tolerate bedsheets. Examination revealed hyperesthesia over lower one-third of both legs and both feet. Knee jerks were present with reënforcement. Absent ankle jerks and no Babinski reflexes. Laboratory studies confirmed a diagnosis of diabetes mellitus. Diabetes controlled and marked relief of neuritic symptoms in four weeks and complete relief in six months.

Case No. 6750 B: A white female, 16 years of age, complained of leg cramps and muscle weakness of her legs. Cramps worse at night and relieved with exercise. Weakness became so marked that at times the patient's knees would completely give out while walking. She thought her leg muscles were getting smaller. One month after onset of these symptoms, patient noticed polydipsia, polyuria, polyphagia and weight loss. Examination showed absent biceps, triceps, knee and ankle jerks. No sensory changes. She was unable to rise from either a squatting or sitting position and walked with great difficulty due to muscle weakness.

Laboratory examination revealed a blood sugar of 375 mgms. %, CO_2 combining power of 20.8 volumes percent, 4+ sugar in the urine and acetonuria.

On diabetic management, the patient made a remarkable recovery and in 4 weeks was able to walk as well as she ever could; was able to rise from sitting position but required assistance to rise from squatting position. At this time the deep reflexes were still absent.

One month later the patient noticed weakness of her hands and arms. Her diabetes was not adequately controlled. With rigid control of her diabetes, she regained strength of her hands and arms. Deep reflexes were still absent. Examination eight months after her first admission showed marked improvement of symptoms. Deep reflexes were still absent and there was still muscle atrophy of the legs.

Case No. 8737 A: A colored female, 59 years of age, with diabetes of two years duration. Complaints of numbness and tingling of hands, feet and lower one-third of legs in addition to weakness, weight loss, polydipsia and polyuria. Patient's diabetes was not well controlled for the past year. Examination showed hypesthesia of hands, feet and lower one-third of both legs. Knee and ankle jerks were markedly diminished. On diabetic management, the numbness and tingling of extremities diminished and in two weeks she was symptom free.

Case No. 76798: A white male, 61 years of age, with essential hypertension and diabetes mellitus of seven years duration, complained of severe pain in right knee, later radiating into right leg, shooting pains in left leg, increasing weakness of legs and burning sensations in both legs. Weakness was more marked in left leg and gradually increased until he was barely able to walk. There was hyperesthesia and tenderness of both legs; weakness of both legs, the left more than the right and diffuse muscle atrophy of both legs. Knee and ankle jerks were absent. There was diminished to absent tactile, pain, thermal and vibratory sensations over the lateral surfaces of the legs, thighs and around the waistline. He was able to stand alone but could not walk. Voluntary movement of the left leg and foot was considerably reduced. Under diabetic management, his symptoms rapidly improved in four weeks and he was able to take a few steps unassisted.

Case No. 44556: A 47 year old colored female with generalized arteriosclerosis and diabetes mellitus of three years duration was admitted for numbness and tingling of hands and feet, feeling of stiffness in chest, hands and feet. Onset of the neurological symptoms began two months previously and the patient's diabetes was poorly controlled since diagnosis was first made. Examination revealed biceps jerks present, absence of right triceps reflex. The knee jerks were hypoactive, the left more than the right. Both ankle jerks were absent. Babinski sign was negative. Blood serology and spinal fluid examinations were negative. Blood sugar on admission was 235 mgm. %. With control of the diabetes, the patient gradually showed relief of her neuritic symptoms at the end of three weeks.

Case No. 6908B: A 64 year old colored female with diabetes mellitus of twenty years' duration and hypertensive heart disease and inability to void for past 4 days before admission was admitted for control of diabetes. At times she was conscious of her bladder distention and at times she was unaware of any bladder distention. No incontinence of bowels or bladder.

Examination showed absence of biceps, triceps, knee and ankle jerks and diminution of vibratory and position senses in the legs. Pelvic examination showed small fibromyomata uterii which were considered by the gynecologist to have no bearing on her inability to void. The bladder was distended to the umbilicus and found to contain 950 cc. residual

urine. Pupils reacted to light. Urological consultants found no pathology of the urinary tract to account for bladder distention. Syphilologist consultant felt her bladder symptoms were due to diabetes and not to her syphilis which was diagnosed at latent syphilis.

Laboratory studies revealed a fasting blood sugar of 527 mgm. %, a CO_2 combining power of 35.6 vol. per cent, a total 24 hour urine sugar of 17 gm., and acetonuria. Blood count revealed 3,960,000 RBC, 11.5 grams of hemoglobin and 9,750 WBC. Differential count showed 76 polymorphonuclear cells, 22 lymphocytes and 2 monocytes. Blood Wassermann was positive. Spinal fluid examination showed a clear fluid, protein 25 mgm., 1 lmphocyte, negative Wassermann and normal Lange, normal pressure and no evidence of block.

The patient was placed on a diabetic diet, 212 grams CHO, 90 grams, protein and 128 grams of fat, and her diabetes was well controlled with 30 units of protamine zinc insulin daily at the end of her third week of hospitalization. By the end of four weeks the patient was able to void small amounts of urine but she still had distention of the bladder. The patient was dismissed with diabetes well controlled.

Nine months later the patient was seen with gangrene of the right foot. On admission her blood sugar was 120 mgm. % and the urine was negative for sugar and acetone. There, was no bladder distention and the patient stated soon after her previous discharge from the hospital, she began to urinate by herself and has had no bladder distention or urinary difficulty since.

The biceps, triceps, knee and ankle jerks were still absent. Position sense and vibratory sense were present in the arms and left leg and foot.

The presence of a positive blood Wassermann in this patient gives rise to the question of whether her bladder disturbance and reflex changes were due to syphilis or diabetes. We have felt the findings were due to diabetes for the following reasons: (1) Normal pupil reactions. (2) Normal spinal fluid. (3) The return of normal bladder function following control of her diabetes. The syphilologist who saw this patient felt no anti-luetic therapy was indicated so the patient received no treatment for her syphilis.

Case No. 25841: A 61 year old colored female with hypertensive heart disease, and diabetes mellitus of 16 years duration. Twelve years after onset of diabetes, the patient complained of weakness of the legs, and numbness and tingling of hands and feet. Diabetes poorly controlled since onset due to extremely poor coöperation of the patient. The patient was admitted in diabetic coma. At this time the patient's bladder was distended and a residual of 700 cc. was found. Two days later bladder retention was still noticed and in addition incontinence of the bowels; at this time the patient was not in acidosis. Other significant findings were absence of vibratory position senses in the feet, numbness and tingling in fingers and feet. There was reduction of pain and touch over

the toes, feet and lower third of both legs: touch was also diminished over the fingers. Marked diminution of the biceps, triceps and radial reflexes was noted and the knee and ankle jerks were bilaterally absent. Negative Babinski.

Blood Wassermann negative. Fractional gastric analysis revealed the presence of 15°, 37°, 25° free HCl at 45 min., 1 hour and 1 hour and 30 minutes respectively. Examination of the blood showed a microcytic hypochromic anemia. There was little improvement in the patient's neurological condition at the end of 8 weeks of satisfactory diabetic management in the hospital.

Admitted one year later and still had incontinence of bowels and bladder and urinary retention. Biceps and triceps jerks diminished and knee and ankle jerks bilaterally absent. Absence of touch and pain still persisted in the feet and lower ⅓ of the legs.

Case No. 5035B: A 28 year old colored male with diabetes mellitus of two years duration, very inadequately controlled during this time, was admitted for generalized sweating, marked weakness in the legs, "grabbing" pains in both legs, chiefly at night. The leg pains began at the hips and radiated downward. Weakness increased until on admission he was unable to walk or stand. These symptoms were of 7 weeks duration.

Examination revealed diminished tendon reflexes in the arms and absent tendon reflexes in both legs. There was marked weakness of the legs bilaterally, especially noticeable in the extensor groups. Hypoesthesia in both feet and legs gradually fading upward and laterally. Deep sensations were intact. Rhomberg negative. No tenderness along nerves.

Two days after admission the patient's weakness showed improvement and he was able to take a few steps. At the end of 16 days of diabetic management the patient's sensations had returned in the legs but were still absent in the feet.

On discharge, five weeks after admission all subjective symptoms were absent and the only signs of neuropathy were absent knee and ankle jerks. When seen two years later he was still free of neuritic symptoms and all deep reflexes were now present.

Case No. 4752 C: A 23 year old colored female with diabetes mellitus of four years duration was admitted for marked weight loss of forty pounds, a persistent diarrhea and an inability to urinate which developed before admission but became more so after being in the hospital, so that she was unable to empty bladder at all. She experienced a marked decrease in the sense of the filling of the bladder. At times she would notice incontinence of the bowls, more so since admission. Bowel incontinence chiefly noted at night. She had never been on insulin.

Examination revealed diminished right biceps reflex, left biceps reflex was normal. The right triceps jerk was absent and the left triceps jerk was barely perceptible. Knee jerks present with reënforcement. Right

ankle jerk was present and the left ankle jerk was absent. The left pupil was slightly irregular and larger than the right. Both pupils showed a sluggish reaction to light as well as a paradonical reaction to light. Reaction to accommodation was normal.

Blood sugar was 555 mgm. %. Urine showed 4+ Benedict's reaction and no acetone. One stool examination was negative for ova and parasites. Blood examination showed RBC 4,020,000, Hb. 12.6 grams and WBC 18,000. Differential count revealed 75 polymorphs, 22 lymphocytes, 2 monocytes and 1 basophile.

Seven months later after being on adequate diabetic control, the bladder control improved remarkably and she was free of urinary incontinence. It was necessary for her to strain to empty the bladder, however. Occasionally she has had residual urine of 100 cc. Diarrhea had still persisted and reflexes were unchanged. Complete studies have revealed diabetes mellitus as the only etiology of the patient's physical findings and symptoms. Blood Wassermann has been negative.

BIBLIOGRAPHY

Angle, F. F.: Tabes Diabetica: Report of a Case., *U.S. Nav. M. Bull.* 26: 81, 1928.

Althaus, Julius: On Sclerosis of the Spinal Cord. New York, Putnam, 1885.

Auche, B.: Des Alterations des nerfs Peripheriques Chez Les Diabetiques, *Arch. de Med. Exper. et D'anat. Path.*, 2:635, 1890.

Bargen, J. A., Bollman, J. L. and Kepler, E. J.: The "Diarrhea of Diabetes" and Steatorrhea of Pancreatic Insufficiency. *Proc. Staff Meet., Mayo Clinic*, 11:737, 1936.

Bolduan, C.: Diabetes, An Important Health Problem. *New Eng. J. Med.*, 206:49, 1932.

Bonardi, E.: Sclerosi Diffusa Pseudo-Sistematizzata del Midollo Spinale con Poli Nevrite in un Caso di Diabete Mellito, *Morgagni*, 39:557, 1897.

Bouchard, Ch.: Sur la Perte des Reflexes Tendineux Dans la Diebete Sucre. *Progress Med.*, 12:819, 1884.

Bowen, B. D. and Aaron, A. H.: Gastric Secretion in Diabetes Mellitus, *Arch. Int. Med.*, 37:674, 1926.

Brain, W. R.: Diseases of the Nervous System, 2nd ed., London, Oxford, Univ. Press, 1940.

Buzzard, T.: Illustrations of Some Less Known Forms of Peripheral Neuritis, Especially Alcoholic Monoplegia, and Diabetic Neuritis. *Brit. M. J.*, 1:1419, 1890.

Bramwell, B.: Diabetes; Perforating Ulcer of the Foot; Advanced Atheroma of the Posterior Fibial Artery, the Artery Being Adherent to the Posterior Fibial Nerve; Marked Changes in the Posterior Tibial and Plantar Nerves, *Clin. Stud.*, 5:279, 1907; quoted by Woltman and Wilder (1929).

Caravati, C. M.: Insulin Neuritis. *Virginia M. Monthly*, 59:745, 1933.

Charcot, M.: Sur un Cas de Paraplegie Diabetique. *Arch. Neurol.*, 19:305, 1890.

Eichhorst, H.: Neuritis Diabetica und Ihre Beziehungen zum Fehlenden Patellarsehmenreflex, *Virchows Arch. F. Path. Anat.*, 127:1, 1892.

Findlay, J. W.: Changes in the Peripheral Nerves in a Case of Diabetes Mellitus, *Tr. Med.-Chir. Soc. Glasgow*, 3:441, 1902.

Fleming, R. A.: Two Cases of Peripheral Neuritis, *Brain*, 20:56, 1897.

Fraser, T. R. and Bruce, A.: Multiple Diabetic Neuritis, *Brit. M. J.*, 1:1149, 1895.

Fraser, T. R. and Bruce, A.: A Case of Diabetic Neuritis With Description of Postmortem Examination of Nerves and Muscles, *Edinburgh M. J.*, 42:300, 1896.

Guttman, L.: Topographic Studies of Disturbance of Sweat Secretion After Complete Lesions of Peripheral Nerves. *J. Neurol. & Psychiat.*, 3:197, 1940.

Hensay, J. Untersuchungen des Central-Nervensystems Bei Diabetes Mellitus, Strassburg, Goiller, 1897, p. 38; quoted by Woltman and Wilder (1929).

Jordan, W. R.: Neuritic Manifestations in Diabetes Mellitus. *Arch. Int. Med.*, 57:307–366, 1936.

Jordan, W. R., Randall, L. O. and Bloor, W. R.: Neuropathy in Diabetes Mellitus; Lipid Constituents of the Nerves Correlated with the Clinical Data. *Arch. Int. Med.*, 55:26, 1935.

Jordan, W. R. and Crabtree, H. H.: Paralysis of the Bladder in Diabetic Patients, *Arch. Int. Med.*, 55:17, 1935.

Joslin, E. P., Root, H. F., White, Priscilla and Marble, Alexander: The Treatment of Diabetes Mellitus. Philadelphia, Lea and Febiger, 7th ed., 1940.

Leichtentritt, H.: Ein Beitrag zur Erkrankung Peripherer Nerven und des Ruckenmarks Bei Diabetes Mellitus, Berlin, G. Schade, 1893, p. 32; quoted by Woltman and Wilder (1929).

Leval-Picquechef: Sur un cas de Paraplegie Diabetique. *Arch. Neurol.*, 19:305, 1890.

Levy, L. K.: Diabetic Neuritis with Especial Consideration of Vitamin B_1. *Tri-State M. J.*, 13:2672, 1941.

Leyden, E.: Die Entzundung der Peripheren Nerven., *Deut Militar. Zeitsch.*, 17:49, 100, 1887.

Lichtheim: (Sandmeyer, W.: Beitrag zur Pathologischen Anatomie des Diabetes Mellitus, *Deutsches Arch. F. Klin. Med.*, 50:381, 1892.)

Major, R. H.: Tabes Diabetica, *J. A. M. A.*, 83:2004, 1924.

Marchal de Calvi: Recherches sur Les Accidents Diabetiques. Paris, P. Asselin, 1864.

Marinesco, M. G.: Cas de Paraplegie Diabetique, *Rev. Neurol.*, 9:719, 1901.

Minor, L.: Etude de L'Etiologie du Tabes, *Arch. de Neurol.*, 17:362, 1889.

McKittrick, L. S. and Root, H. F.: Diabetic Surgery. Philadelphia, Lea and Febiger, 1928.

Naunyn, B.: Der Diabetes Mellitus, in Nothnagel, H.: Specialle Pathologie und Therapie, Vienna, A. Holder, 1906, Vol. 7.

Needles, Wm.: Vitamin B_1 Therapy in Diabetic Neuritis. *J.A.M.A.*, 121:914, 1943.

Nesbit, R. M. and Gordon, Wm. G.: The Neurogenic Bladder. *Pennsylvania M. J.*, 43:1261, 1940.

Nonne, M.: Ueber Poliomyelitis Anterior Chronica als Ursache Einer Chronisch-progressiven Atrophischen Lahmung Bei Diabetes Mellitus, *Berl. Klin. Wchnschr.*, 33:207, 1896.

Ossokine, N.: Contribution a L'Etude de L'Anatomie Pathologique de la Moelle dans le Diabete Sucre, Abstr., *Rev. Neurol.*, 10:993, 1902.

Pavy, F. W.: Introductory Address to the Discussion on the Clinical Aspect of Glycosuria. *Lancet*, 2:1085, 1885.

Peters, R. A.: The Biochemical Lesion in Vitamin B_1 Deficiency. *Lancet*, 1:1161, 1936.

Pryce, T. D.: Perforating Ulcers of Both Feet Associated with Diabetes and Ataxic Symptoms. *Lancet*, 2:11, 1887.

Pryce, T. D.: Diabetic Neuritis with a Clinical and Pathological Description of Three Cases of Diabetic Pseudotabes, *Brain*, 16:416, 1893.

Quastel, J. H.: Metabolism of Brain and Nerve. *Ann. Rev. Biochem.*, 8:435, 1939.

Robinson, W. D.: Melnick, D. and Field, Henry, Jr.: Urinary Excretion of Thiamin in Clinical Cases and the Value of Such Analyses in the Diagnosis of Thiamin Deficiency. *J. Clin. Invest.*, 19:399, 1940.

Root, H. F.: Rare Paralyses in Diabetes Mellitus. *M. Clin. North Am.*, 5:1433, 1922.

Rooth, H. F. and Rogers, M. H.: Diabetic Neuritis with Paralysis, *New Eng. J. Med.*, 202:1049, 1930.

Rudy, A. and Muellner, S. R.: The Neurogenic Bladder in Diabetes Mellitus: Early Recognition and Treatment with a Report of Cases. *J. Urol.*, 45:844, 1941.

Rundles, R. Wayne: Diabetic Neuropathy. *Med.*, 24:111–160, 1945.

Schweiger, L.: Ueber Die Tagiformen Veranderungen der Hinterstrange Bei Diabetes, *Wien. Med. Wchnschr.*, 57:1549, 1907.

Severinghaus, E. L.: Study of 500 Diabetics. *Am. J. Med. Sc.*, 182:311, 1931.

Shumacker, H. B.: Sympathetic Denervation of the Feet and Legs Occurring Spontaneously or as a Result of Disease. *Bull. Johns Hopkins Hosp.*, 71:1, 1942.

Souques, A. and Marinesco, G.: Lesions de la Moelle Epiniere Dans un Cas de Diabete Sucre, *Rev. Neurol.*, 5:242, 1897.

Stadie, W. C.: Intermediary Metabolism in Diabetes Mellitus. *Bull. N. Y. Acad. Med.*, 19:778, 1943.

von Leyden, E. and Goldscheider: Die Erkrankungn des Ruckenmarks und der Medulla Oblongata, in Nothnagel, H.: Specielle Pathologie und Therapie, Vienna, A. Holder, 1895, vol. 10.

von Leyden, E.: Beitrage zur Klinik des Diabetes Mellitus, *Wien. Med. Wchnschr.*, 43:926, 1893.

Warren, S.: Pathology of Diabetes, Philadelphia, Lea & Febiger, 1930.

White, Priscilla: Diabetes in Youth. *N. Eng. J. Med.*, 224:586, 1941.

Wilder, Russell M.: Clinical Diabetes Mellitus and Hyperinsulinism. Philadelphia, Saunders, 1940, p. 383.

Wilkins, Robert, and Kolb, L. C.: Vasomotor Disturbances in Peripheral Neuritis. *Am. J. Med. Sc.*, 202:216, 1941.

Williamson, R. T.: On the Knee Jerks in Diabetes Mellitus, *Lancet*, 2:138, 1897.

Williamson, R. T.: Changes in the Spinal Cord in Diabetes Mellitus, *Brit. M. J.*, 1:122, 1904.

Williamson, R. T.: Diabetic Neuritis, *Practitioner*, 112:85 (Feb.) 1924.

Wittmaack, K.: Degenerative Neuritie Bei Diabetes Mellitus, *Ztschr. F. Ohrenh*, 53:19, 1907.

Woltman, H. W. and Wilder, R. M.: Diabetes Mellitus, Pathologic Changes in the Spinal Cord and Peripheral Nerves. *Arch. Int. Med.*, 44:576, 1929.

Woltman, H. W. and Wilder, R. M.: Diabetes Mellitus: Pathologic Changes in the Spinal Cord and Peripheral Nerves, *Arch. Int. Med.*, 44:574, Oct. 1929.

Wright, I. S.: Hereditary and Familial Diabetes Mellitus, *Am. J. M. Sc.*, 182:484, 1931.

CULTIVATION OF ENDOMETRIUM *IN VITRO*— A PRELIMINARY NOTE†

J. R. VALLE* AND C. M. POMERAT

In the course of experiments dealing with the effect of hormones on various tissues cultivated *in vitro*, simple hanging drop preparations were made of guinea pig endometrium. While successful cultures of this tissue have been reported by various workers, the simplicity of the method for achieving successful outgrowths suggested the advisability of calling attention again to this important preparation for assay purposes. A review of the literature together with a more extensive study of endometrial tissue *in vitro* is in progress.

Adult non-pregnant guinea pigs were given five daily doses of 0.5 ml. of a solution of Di-ovocylin (Ciba) containing 50 rat units per ml. Untreated animals were used as controls.

Uteri were removed aseptically and transferred to sterile petri dishes. The endometrium was stripped from the underlying tissues by means of forceps. It should be noted that this procedure is relatively difficult in untreated animals. Endometrial fragments were cut approximately one millimeter square and transferred to a watch glass containing Tyrode's solution until ready for culture. One drop of equal parts of chick embryo juice (7-day incubation) and of rabbit serum was placed on a cover slip. After transferring one endometrial fragment, the mixture was clotted with a drop of heparinized rooster plasma. The serum was derived from (a) rabbits on the fifteenth day of pregnancy and (b) normal male rabbits (controls).

Day-to-day observations were made on living cultures and at 96 hours they were fixed one hour in Helly-Zenker's, washed thoroughly and stained overnight in dilute Delafield's hematoxylin.

---

†From the Tissue-Culture Laboratory, Department of Anatomy, The University of Texas Medical Branch, Galveston. Received January 8, 1947.

*Guggenheim Fellow; Professor of Pharmacology, University of Sao Paulo.

Results were based on the study of four preparations of each of four experimental conditions. (1) *No estrin treatment. Serum from male rabbit in the medium.* Very few spindle cells but generally poor outgrowth. (2) *No estrin treatment. Serum from pregnant rabbit in the medium.* Spindle fiber outgrowth. One preparation (Fig. 1) showed a moderately heavy pattern of interlacing cells suggestive of a stromal reaction. (3) *Estrin treated animal. Serum from male rabbit in the medium.* Moderate outgrowth of epithelial cells. No spindle fibers present. (4) *Estrin treated animal. Serum from pregnant rabbit in the medium.* Good outgrowth of epithelial cells (Fig. 2). Mitotic figures numerous throughout the sheet of migrating cells (Fig. 3).

Uniformly poor outgrowth in another group of cultures prepared in the same manner but containing human pregnant and non-pregnant serum suggested the presence of a factor inhibitory to guinea pig endometrium in the medium used.

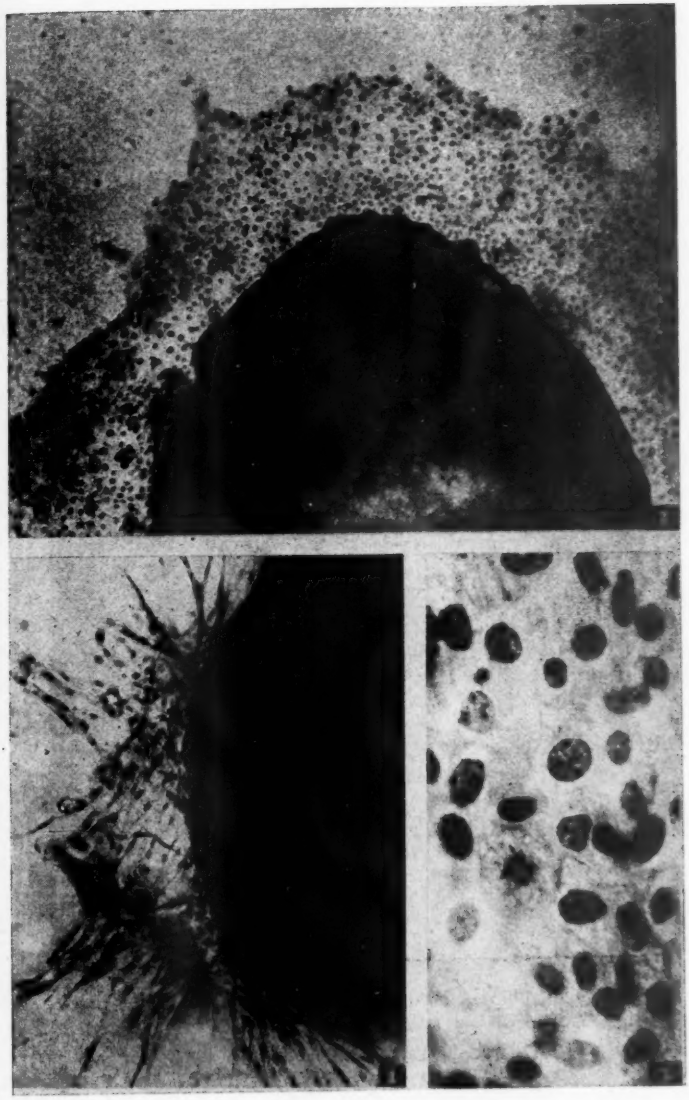
This brief description of a method for the *in vitro* cultivation of endometrium affords an opportunity for endocrinologists to undertake extensive and systematic study of changes produced in the endometrium under controlled conditions with various naturally acting hormones. It also provides an important possibility for assay purposes.

Figures 1–3. Guinea pig endometrium cultivated in vitro for 96 hours in a medium containing 25% pregnant rabbit serum. Fixed in Helly-Zenker's solution and stained in Delafield's hematoxylin.

Figure 1. Spindle cell outgrowth from an endometrial fragment of animal not previously treated with estrogen. 80X.

Figure 2. Epithelial sheet migrating from an endothelial fragment of an animal treated with 5 daily doses of 0.5 ml. Di-ovocylin (Ciba) containing 50 r. u. per ml. 80X.

Figure 3. Same as Fig. 2 but at 500X.



CALLING ATTENTION TO:

Items of Possible Interest to Friends of Our Effort

Welcome to 1947

1. New Books: *The United States Quarterly Book List* (Library of Congress, $1.75 per vol. from Supt. Documents, U.S. Gov. Printing Office, Washington 25) offers selected pertinent reviews with biographical data. Sir Charles Sherrington is keen as ever in *The Endeavor of Jean Fernel* (Macmillan, N.Y., '46, $3.50). L. I. Dublin and A. J. Lotka revise important *Money Value of a Man* (Ronald Press, N.Y., 205 pp. '46, $6). J. Dobson offers useful *Anatomical Eponyms* (Bailliere, London, '46, 249 pp., $7). W. Klyne issues a *Practical Chemistry for Medical Students* with fundamental scientific orientation (Livingstone, Edinburgh, '46, 474 pp., $5). W. S. Craig documents well in *Child and Adolscent Life in Health and Disease: A Study in (British) Social Pediatrics* (Livingstone, Edinburgh, '46, 683 pp., $6). T. Reik presents *Ritual Psychoanalytic Studies* (Farrar Straus, N.Y., '46, $5). J. P. Baxter gives official story of O.S.R.D. (in *Scientists Against Time* (Little Brown, Boston, 488 pp., $5). J. P. Greenstein announces important *Biochemistry of Cancer* (Academic Press, N.Y., '47, $7). G. Pincus edits *Recent Progress in Hormone Research* (Academic Press, N.Y., '47, 400 pp., $7). J. Barcroft summarizes *Researches on Pre-Natal Life* (Blackwell's, Oxford, '46, $9). R. Crawshay-Williams discusses *The Comforts of Unreason* (Blackwell's, Oxford, '46, $4). H. J. Eysenck describes *Dimensions of Personality* (Blackwell's, Oxford, '46, $6). Symposium appears on *Heredity and Variation in Microorganisms* (Cold Spring Harbor Biological Lab., N.Y., '46, 300 pp., $6). C. E. K. Mees reveals *The Path of Science* (Wiley, N.Y., '46, 240 pp., $3). New symposium appears on *Dental Caries and Fluorine* (A.A.A.S., Washington, 119 pp., '46, $3.50). E. T. Engle edits *The Problem of Fertility* (Princeton Press, '46, 320 pp., $3.75). A. Magitot is stimulating in *Physiologie Oculaire Clinique* (Masson, Paris, '46, 750 fr.).

2. Pharmacology: Curare gets well reviewed by E. B. Schlesinger (*Am. J. Med.* 1: 518/46) and by A. E. Bennett (*Am. Scientist* 34: 424/46). J. H. Comroe and Co. find di-isopropyl fluorophosphate (DFP) inferior to neostigmine in myasthenia gravis (*Am. J. Med. Sci.* 212: 641/46). C. Heymans and Co. report dibenzyl methylamine is diuretic and central stimulant (*Arch. Internat. Pharmaco Therap.* 72: 233/46). D. I. Macht finds thromboplastic effects from digitalis and mercurial diuretics, offering possible explanation of sudden deaths in high dosage (*Ibid.* p. 297). R. M. Suarex, A. D. Welch and Co. find conjugated pteroylglutamic (folic) acids increase urinary output of folic acid, which is further increased by folic acid free liver extract (*J. Lab. Clin. Med.* 31: 1294/46). R. K. Richards and G. M. Everett summarize data on tridione (*Ibid.* p. 1330). J. Levy and Co. study effects of central stimulant and depressant drugs on brain

slice respiration (*Bull. Soc. Chim. Biol.* 28: 328–341/46). E. B. Verney discusses absorption and excretion of water in relation to antidiuretic hormone (*Lancet* 2: 744, 781, Nov. 23, 30/46). T. S. Gardner finds daily ingestion of yeast nucleic acid prolongs average life span of mice (*J. Gerontol.* 1: 445/46), confirming T. B. Robertson (*Austral. J. Exp. Biol. Med.* 5: 47/28).

3. CHEMOTHERAPY: Excellent symposium on background to chemotherapy (*Brit. Med. Bull.* 4: 241–289/46) with history by E. M. Lourie, microbial antagonisms by H. W. Florey, antibacterial action by H. McIlwain, nutrition of bacteria by P. Fildes, microbial resistance by F. R. Selbie, chemotherapy of rickettsial and virus diseases by C. H. Andrews and H. Kirby, local chemotherapy by L. P. Garrod, and principles of administration by J. H. Gaddum: also review of chemotherapy from Institute Pasteur, Paris, by the J. Trefouels, D. Bovet and F. Nitti. R. A. Peters, R. H. S. Thompson and L. A. Stocken report on mechanism of arsenic toxicity by interference with pyruvate oxidase systems with protection by dithiols (*Biochem. J.* 40: 513–554/46). R. H. Major reviews favorable results with nisulfasole in chronic ulcerative colitis (*Am. J. Med.* 1: 485/46). A. M. Fisher describes bacteriocidal penicillin action on bacterial morphology (*J. Bact.* 52: 539/46). G. W. Irving and Co. report fungicidal action of antibiotic tomatin (*Ibid.* p. 601). Excellent symposium on antibiotics including microbiological and pharmacological discussion by 20 contributors (*Ann. N.Y. Acad. Sci.* 48: 31–218/46). C. Levaditi and Co. propose liposoluble combination of 30,000 U. methyl penicillin and 100 mgms. bismuth octyl ethylmalonate for effective syphilis therapy (*Presse Med.* 57: 781, Nov. 23/46).

4. FURTHERMORE: G. M. Lyon discusses atomic energy and future health (*Milit. Surg.* 99: 762/46). L. Nanninga shows fibrinogen with molecular weight around 400,000 is no protease (*Arch. Neerl. Physiol.* 27: 241/46). R. Jakowicz and L. M. Bryce find cerebro-spinal fluid inhibits Rh agglutinins (*Med. J. Austral.* 2: 740, Nov. 23/46). H. Henry and M. Stacey show gram-positive or dye retaining constituent of cell is nucleoprotein (*Proc. Roy. Soc. B.* 133: 391/46). Our C. R. Allen and H. C. Slocum recommend anesthetic control with cyclopropane in Cesarean section (*Texas S. J. Med.* 42: 482/46).

FEBRUARY, 1947

1. MORE BOOKS: Interesting biographies include: A Flexner's *Daniel Coit Gilman, Creator of the American Type of University* (Harcourt, Brace, N.Y., '64, 182 pp., $2), S. Harris's *Banting's Miracle, the Story of the Discoverer of Insulin* (Lippincott, Phila., '46, 265 pp., $3) & J. A. Lomax's *Adventures of a Ballad Hunter* (Macmillan, N.Y., '47, 309 pp., $3.75). Also A. H. Maloney's *Amber Gold* (Meador, Boston, '46, 448 pp., $2.50). H. Ward edits popular *New Worlds in Medicine* (McBride, N.Y., '46, 707 pp., $5). L. Bryson discusses *Science and Freedom* (Columbia

Univ. Press, N.Y., '46, 251 pp., $2.75). G. Lawton offers *Aging Successfully* (Columbia Press, N.Y., '47, 266 pp., $2.75). C. Gruchy notes *Creative Old Age* (Counseling Center, San Francisco, '46, 143 pp., $2.75). M. F. A. Montagu reports on *Adolescent Sterility* (C. C. Thomas, Springfield, Ill., '46, 157 pp., $3.50). M. Luckiesh discusses *Applications of Germicidal, Erythemal & Infrared Energy* (Van Nostrand, N.Y., '46, 470 pp., $5.50). F. J. Hodges, I. Lampe & J. F. Holt offer useful *Radiology for Medical Students* (Year Book Publ., Chicago, '47, 411 pp., $6.75). E. H. Hashinger completes Logan Clendening's posthumous *Methods of Diagnosis* (Mosby, St. Louis, '47, 1064 pp., $10). J. E. M. Thomson's *Orthopedic Problems* appears (Edwards, Ann Arbor, '47, 234 pp., $6). R. A. Cooke offers *Allergy* (Saunders, Phila., '47, 572 pp., $8). H. H. Horner surveys *Dental Education Today* (Univ. Chicago Press, '47, 454 pp., $6). L. Daniels, M. Williams, & C. Worthingham describe *Muscle Testing* (Saunders, Phila., '47, 189 pp., $2.50). D. E. Lea notes *Actions of Radiation on Living Cells* (Macmillan, N.Y., '47, 311 pp., $4). E. D. P. de Robertis, W. W. Nowinski & Saez offer excellent *Citologia General* (El Ateneo, Buenos Aires, '46, $4.50).

2. Cancer: Russian symposium includes L. A. Silber on filtrability of tumors; A. D. Timofeyski on tumors in tissue culture; G. Roskin on toxin therapy of cancer & toxicity of blood in cancer, & cancerolytic substance of Trypanosoma Cruzi, & A. Y. Altgauzen on diagnosis of malignancy from fresh smears (*Amer. Rev. Soviet Medicine* 4: 100–154, '46). E. V. Cowdry proposes financing of cancer research (*Science* 105: 53 Jan. 17/47). A. R. Gopal-Ayenga & E. V. Cowdry report drop in desoxyribose nucleic acid in chromosomes after methylcholanthrene with marked rise when carcinoma develops (*Cancer Res.* 7: 1/47). C. Carruthers & V. Zuntzeft note increase in activity of succinic dehydrogenase & cytochrome oxidase in cancerous tissue (*Ibid.* 9). See abstracts of '46 Gibson Island Conference on cancer (*Ibid.* p. 37). F. R. Miller & Co. note remissions in lymphoblastic leukemia following injections of crude myelokentric acid from urine of chronic myeloid leukemia patients (*Blood* 2: 15/47).

3. Acetylcholine: E. J. Carey & Co. say that motor end plates in muscle seem to be biologic jet pumps to discharge acetylcholine into muscle myoplasm (*Amer. J. Path.* 22: 1205/46). C. S. Koschtojanz & T. M. Turpajew find –SH necessary for acetylcholine effects on heart (*Nature* 158: 837, Dec. 7/46). B. Minz (Paris) suggests that thiamin in cocarboxylase is factor in acetylcholine synthesis (*Proc. Soc. Exp. Biol. Med.* 63: 280/46). J. E. Davis finds that pteroyl glutamic acid increases cholinesterase activity of sera (*Ibid.* p. 287).

4. Also: Nobelate H. J. Muller gives Pilgrim Trust Lecture on gene (*Proc. Roy. Soc. B.* 134: 1/47). J. A. Ryle well discusses meaning of normal (*Lancet* 1:1 Jan. 4/47). G. Ekehorn criticises H. W. Smith's renal kinetic theory (*Acta Med. Scand.* 124: 282, 379; 125: 148/46). George Dock reminisces pleasantly on clinical pathology in the eighties & nineties (*Am. J. Clin. Path.* 16: 671/46). M. J. Andre discusses oxygen

apnoea as noted by A. Mosso (*Arch. Ital. Biol.* 41: 138, 157, 1904; Presse Med. 60: 815, Dec. 7/46). A. Leya describes marked antianaphylactic action of dimethylamino ethyl-benzylaniline HCl ("antergan") (*Compt. Rend. Soc. Biol.* 140: 191/46). J. H. Lawrence & Co. note convulsive narcosis in mice from 60% xenon in oxygen & give oil-water solubilities for inert gases (J. Physiol. 105: 197/46). See symposium on thyreotoxicosis (*Med. J. Austral.* 2: 789–809, Dec. 7/46). B. M. Bloomberg reports on absorption & excretion of p-amino benzoic acid (*S. Afr. J. Med. Sci.* 11: 51/46). E. Boyland notes acute convulsive effects from water reaction compounds of nitrogen mustards (*Brit. J. Pharmacol.* 1: 247/46). F. M. Berger & W. Bradley find powerful safe muscle relaxation from dihydroxy methyl phenoxy propane ("myanesin") (*Ibid.* p. 265). J. C. Trent's interesting biography of yellow fever runs through year's issues of *N. Carolina Med. J.* (7: 22, etc., 654/46). A. Cournand analyzes dynamics of pulmonary circulation (*Bull. N.Y. Acad. Med.* 23: 27/47). D. E. Lea & M. H. Salaman suggest that smaller bacteriophages are macromolecules like crystalline plant viruses while larger phages are primitive single celled organisms (*Proc. Roy. Soc. B.* 133: 434/46). H. Fraenkel-Conrat & Co. find succinic ester of gramacidin active & nontoxic (*Proc. Soc. Exp. Biol. Med.* 63: 302/46). Our C. M. Pomerat & Co. describe toxicity of barbiturates on tissue cultures (*Ibid.* p. 322).